PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Michael A. Apicella et al.

Serial No.: 09/077,572

Filed: October 13, 1998

For: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Examiner: S. Devi

Group Art Unit: 1645

Docket: 875.001US2

APPELLANTS' BRIEF ON APPEAL

Box AF
Commissioner for Patents
Washington, D.C. 20231

Sir:

This Brief is presented in support of the Notice of Appeal mailed August 17, 2001 and filed in the U.S. Patent and Trademark Office on August 27, 2001, from the final rejection of claims 22-26, 29, 32 and 33 of the above-identified application, as set forth in the final Office Action mailed September 20, 2000. Appellants request an oral hearing.

This Brief is being submitted in triplicate, as set forth in 37 C.F.R. § 1.192(a).

A Petition for Extension of Time, with authorization to charge the fee to Deposit Account No. 19-0743, is enclosed.

The Commissioner is hereby authorized to charge the brief filing fee of $320.00 and request for oral hearing fee of $280.00, and any other fees which may be due, and to credit any overpayments, to Deposit Account No. 19-0743.

APPELLANTS' BRIEF ON APPEAL

TABLE OF CONTENTS

1. REAL PARTY IN INTEREST

The real party in interest of the above-captioned patent application is the assignee, University of Iowa Research Foundation.

2. RELATED APPEALS AND INTERFERENCES

The Appellants, their legal representatives, and the assignee are not aware of any other appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

3. STATUS OF THE CLAIMS

For the purpose of this appeal, claims 22-26, 29, 32 and 33 stand rejected. Claims 22-26, 29, and 32-34 are the subject of this appeal (*see* Appendix I).

4. STATUS OF AMENDMENTS

Appellants on March 16, 2001 filed by facsimile a Response indicating the ATCC deposit numbers for nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29. A copy of this document is enclosed in Appendix II. Appellants did not receive an Advisory Action with respect to this submission. Therefore, Appellants presume, for the purpose of this appeal, that this amendment was not entered.

Enclosed herewith is a Response amending the specification to indicate the ATCC deposit numbers for nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29. The enclosed Response also provides amendments to claims 22 and 29, and newly added claim 34. Claims 24 and 26 have been amended to recite a mutant endotoxin that is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A. Claim 34 recites in dependent form an element of previously pending claim 22.

5. SUMMARY OF THE INVENTION

Gram-negative bacteria have an outer membrane comprised of proteins, lipoproteins, phospholipids, and glycolipids. The glycolipids comprise primarily endotoxin lipopolysaccharides (LPS) or lipooligosaccharides (LOS), depending on the genus of bacteria.. Specification at page 1, lines 21-26. LPS and LOS have potential as vaccines because of the antigenic determinants ("epitopes") residing in their carbohydrate structures. The chemical nature of LPS and LOS prevent the use of these molecules in vaccine formulations, because of the inherent toxicity of the lipid A portion. Accordingly, there are no currently available endotoxins that can safely be used as effective vaccines, *i.e.*, can induce an antibody response to these LPS or LOS antigenic epitopes. Specification at page 2, lines 21-35.

The present claims are directed to a method of making a mutant endotoxin, a mutant endotoxin made by this method, and a method of producing endotoxin-specific antisera, where the endotoxin has substantially reduced toxicity as compared to the wild-type endotoxin. Structurally, the endotoxin recited in the present claims is the same as the wild type endotoxin, except that it lacks at least one secondary acyl chain on lipid A.

6. ISSUE PRESENTED FOR REVIEW

1. Whether the specification provides adequate enablement under 35 U.S.C. § 112, first paragraph, for pending claims 22-26, 29 and 32-33.

7. GROUPING OF CLAIMS

The following grouping of claims is made in compliance with the requirements of 37 C.F.R. § 1.191 for the content of an Appeal Brief. The following grouping of claims is made to expedite this appeal and to narrow the issues, and is not intended to waive or limit the right of the Appellants to enforce and defend claims separately, even though they are grouped for convenience in this Appeal. For the purpose of this appeal all the pending claims (claims 22-26,

29 and 32-34) stand or fall together. All of the pending claims recite a mutant endotoxin, or make or use a mutant endotoxin, that (1) functionally has substantially reduced toxicity as compared to an endotoxin of a wild-type bacterial pathogen of the same species as the mutant pathogen, and (2) structurally is the same as wild type endotoxin except for lacking at least one secondary acyl chain on lipid A.

8. ARGUMENT

A. Applicable Law: 35 U.S.C. § 112, first paragraph

The first paragraph of 35 U.S.C. § 112 states:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

To be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. *Genentech Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 42 U.S.P.Q.2d (BNA) 1001, 1004 (Fed. Cir. 1997).

1. Patent Office's Position

The Patent Office has taken the position that the specification fails to provide an enabling disclosure for the pending claims. The Patent Office acknowledges that Appellants have submitted a copy of the ATCC deposit receipt showing that the proper strains have been deposited under the provisions of the Budapest Treaty and provided the proper statement that all restrictions will be irrevocably removed upon the granting of a patent in compliance with 37 CFR 1.801-1.809. The Patent Office, however maintained the enablement rejection because Appellants in advertently provided the incorrect location in the specification into which the deposit information was to be inserted.

2. Appellants Response

Appellant encloses herewith a Response to the Office Action that indicates that the specification is to be amended at page 13 to recite that "Nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29 were deposited on November 14, 2000 with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209 under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application. Strain B28 has been accorded accession number PTA-2667 and strain B29 has been accorded accession number PTA-2668." Therefore, this rejection under 35 U.S.C. § 112, first paragraph should be withdrawn.

9. SUMMARY

Each of the pending claims subject to this appeal (claims 22-26, 29, 32-34) is patentable, and in particular, meets the requirements of 35 U.S.C. § 112, first paragraph). Reversal of the rejection and allowance of the claims is appropriate and is respectfully requested.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.
By their Representatives,
SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 15 October 2001 By _____
Ann S. Viksnins
Reg. No. 37,748

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: BOX AF, Commissioner of Patents, Washington, D.C. 20231, on this 15th day of October, 2001.

Candis [illegible]nding
Name

_____
Signature

APPENDIX I

The Claims on Appeal

22. A method of making a mutant endotoxin comprising

mutating an *htrB* gene encoding a wild type endotoxin in a wild type gram-negative bacterial pathogen to provide the mutant endotoxin; wherein the mutant endotoxin is the same as the wild type endotoxin except for lacking one or more secondary acyl chains of lipid A, and wherein the mutant endotoxin has substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen.

23. A mutant endotoxin made according to the method of claim 22, wherein the mutant endotoxin was purified from the *htrB* mutant pathogen by phenol-water extraction or by protease digestion.

24. The mutant endotoxin according to claim 23, wherein the mutant endotoxin is conjugated to a carrier protein.

25. A mutant endotoxin made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, wherein the mutant endotoxin is conjugated to a carrier protein.

29. A method for producing endotoxin-specific antisera, the method comprising

(a) immunizing an individual with a vaccine formulation comprising an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, or endotoxin purified from the *htrB* mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the *htrB* mutant endotoxin is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A.

32. The method of claim 22 wherein the gram-negative bacterial pathogen is of the genera *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella* or *Pseudomonas*.

33. The method of claim 29 wherein the gram-negative bacterial pathogen is of the genera *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella* or *Pseudomonas*.

34. The method of claim 22, further comprising the step of purifying the mutant endotoxin.

APPENDIX II

Office Actions and Amendments

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/171,572	10/13/98	APICELLA M	375061US2

HM12/0301
SCHWEGMAN LUNDBERG WOESSNER & KLUTH
PO BOX 2938
MINNEAPOLIS MN 55402

EXAMINER
DEVI,S

ART UNIT	PAPER NUMBER
1641	5

DATE MAILED: 03/01/99

DV April 1, 1999
August 1, 1999

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

RESTRICTION REQUIREMENT
FILE DIVISIONAL APPLN?

RESTRICTION REQUIREMENT
FILE DIVISIONAL APPLN?

Office Action Summary	Application No. 09/077,572	Applicant(s) Apicella et al.
	Examiner S. Devi, Ph.D.	Group Art Unit 1641

☒ Responsive to communication(s) filed on *Oct 13, 1998*.

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *one* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-29* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☐ Claim(s) _____ is/are rejected.

☐ Claim(s) _____ is/are objected to.

☒ Claims *1-29* are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

Election/Restriction

1) **Please Note**: In an effort to enhance communication with our customers and reduce processing time, Group 1640 is running a Fax Response Pilot for Written Restriction Requirements. A dedicated Fax machine is in place to receive your responses. The Fax number is 703-305-3704. A Fax cover sheet is attached to this Office Action for your convenience. We encourage your participation in this Pilot program. If you have any questions or suggestions please contact Donald E. Adams, Ph.D., Supervisory Patent Examiner at Donald.Adams@uspto.gov or 703-308-0570. Thank you in advance for allowing us to enhance our customer service. Please limit the use of this dedicated Fax number to responses to Written Restrictions.

2) Restriction to one of the following inventions is required under PCT Rule 13.1 and 13.2:

I. Claims 1-21, 27 and 28, drawn to a vaccine formulation comprising an *htrB* mutant of a gram-negative bacterial pathogen, a method of making the mutant and a method of immunizing using the vaccine, classified in class 424, subclasses 93.4 and 184.1

II. Claims 22-26 and 29, drawn to a mutant endotoxin of reduced toxicity, a method of making the mutant endotoxin and a method of producing endotoxin-specific antisera, classified in class 536, subclass 123.1 and class 435, subclass 101.

3) The special technical feature of invention I is a whole cell mutant bacterium, a method of making the mutant and a method of using the mutant as a vaccine. The special technical feature of invention II is a mutant endotoxin, a method of making it and a method of using it for producing specific antisera. Individually, these are permitted combinations of categories under PCT Rule 13.2. Inventions I and II are clearly drawn to two distinct products: a whole cell bacterium and an endotoxic component of a bacterium, each with specific and independent utility. Both products can have independent therapeutic and non-therapeutic, prophylactic and non-prophylactic, and diagnostic utilities in *in vitro* assays for example. As therapeutic or prophylactic components, these products can elicit distinct biologic and immunologic effects. The special

technical features of the two inventions are not so linked because the endotoxin of invention II can be synthesized independently without the use of the bacterium of invention I, for example, by a chemical synthetic process. The bacterium of invention I and the endotoxin of invention II can be used for a materially different process of using the products such as, in an *in vitro* diagnostic assay as sources of coating antigens.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classifications/subclassifications and divergent subject matter, restriction for examination purposes as indicated is proper.

4) Applicants are advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

5) Applicants are reminded that upon cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filled petition under CFR 1.48(b) and by the fee required under 37 CFR 1.17(h).

6) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi whose telephone number is (703) 308-9347. The Examiner can normally be reached on Monday to Friday from 7.45 am to 4.15 pm. A message may be left on Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, James Housel, can be reached on (703) 308-4027. The fax phone number for this Group is (703) 305-7939.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

February 1999

James C. Housel
JAMES C. HOUSEL 2/26/99
SUPERVISORY PATENT EXAMINER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al.

Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Docket No.: 875.001US2
Filed: June 1, 1998
Examiner: S. Devi

Serial No.: 09/077,572
Due Date: April 1, 1999
Group Art Unit: 1641

Assistant Commissioner for Patents
Washington, D.C. 20231

We are transmitting herewith the following attached items (as indicated with an "X"):

X A return postcard.
X Response to Restriction Requirement (1 Page).

Please consider this a PETITION FOR EXTENSION OF TIME for sufficient number of months to enter these papers and please charge any additional required fees or credit overpayment to Deposit Account No. 19-0743.

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this Transmittal Letter and the paper, as described above, are being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 31st day of March, 1999.

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)

By: _____
Atty: Ann S. Viksnins
Reg. No. 37,748

(GENERAL)

S/N 09/077,572 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1641
Filed:	June 1, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

RESPONSE TO RESTRICTION REQUIREMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

In response to the Restriction Requirement mailed March 1, 1999, Applicants provisionally elect, with traverse, Group II (claims 22-26 and 29). Applicants respectfully cancel remaining claims 1-21, 27 and 28 (Group I) without prejudice, and reserve the right to reintroduce them in a divisional application at a later date.

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number if there are any questions regarding this Response or if prosecution of this application may be assisted thereby.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 31 March 1999 By _____

Ann S. Viksnins
Reg. No. 37,748

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents, Washington, D.C. 20231 on March 31, 1999.

Ann S. Viksnins
Name

_____
Signature

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/077,572	10/13/98	APICELLA M	875001052

HM12/0428

SCHWEGMAN LUNDBERG WOESSNER & KLUTH
PO BOX 2938
MINNEAPOLIS MN 55402

EXAMINER	
DEVI,S	

ART UNIT	PAPER NUMBER
1641	11

DATE MAILED: 04/28/99

July 28, 1999
Oct. 28, 1999

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/077,572	Apicella et al.
Examiner	**Group Art Unit**
S. Devi, Ph.D.	1641

☒ Responsive to communication(s) filed on *Apr 5, 1999*.

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *22-26 and 29* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☒ Claim(s) *1-21, 27, and 28* is/are cancelled.

☒ Claim(s) *22-26 and 29* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☒ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *8*

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

1) The instant application has been filed as a national stage 371 application of the PCT application, PCT/US96/18984, filed 11/27/1996 with a priority claim to the national application, SN 08/565,943, filed 12/01/95 in the USA. However, the priority status is not provided in the first paragraph of the instant specification.

Amendment

2) Acknowledgment is made of Applicants' preliminary amendment filed 10/13/98 (paper no. 7). With this, Applicants have replaced the original page 69 with the new page 69.

Information Disclosure Statement

3) Acknowledgment is made of Applicants' Information Disclosure Statement filed 11/16/98 (paper no. 8). The information referred to therein has been considered and a signed copy is attached to this Office Action (paper no. 11).

Election

4) Acknowledgment is made of Applicants' election, without traverse, of Invention II, claims 22-26 and 29, filed 05 April 1999 (paper no. 10).

Claims Status

5) Applicants have canceled the non-elected claims 1-21, 27 and 28 through paper no. 10.

Elected claims 22-26 and 29 are pending in this application and are under examination. An Action on the Merits for these claims is issued in the instant Office Action.

Drawings

6) The drawings are objected to under 37 CFR 1.84 because of the reasons set forth by the Draftsperson in the attached Form PTO 948 (paper no. 11). Correction is required.

Specification/Informalities

7) The specification of the instant application is objected to because:

(a) The first paragraph of the instant specification does not disclose the priority status. The priority status of the instant specification needs to be amended to include the prior applications to which priority is claimed.

(b) The recitation "acylxyacyl" hydrolase is not understood on page 12, line 13. Clarification is required.

Double Patenting

8) The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970) and *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

9) Claims 22, 23, 25 and 29 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 19, 20 and 22 of the copending Application, SN 08/565,943. Although the conflicting claims are not identical, they are not patentably distinct from each other. The method of making in the bacteria of the genera *Haemophilus* or *Neisseria* an endotoxin of substantially reduced toxicity using a *htrB* mutant, an endotoxin of substantially reduced endotoxicity purified from the *htrB* mutant and generation of antibodies to such an endotoxin claimed in claims 19, 20 and 22 of the copending Application are encompassed by the method of making in a Gram negative bacterial pathogen an endotoxin of substantially reduced toxicity using a *htrB* mutant, a mutant endotoxin of substantially reduced endotoxicity purified from the *htrB* mutant and a method of producing endotoxin-specific antisera claimed in claims 22, 23, 25 and 29 of the instant application.

This is a provisional obviousness-type double patenting rejection because the conflicting

claims have not in fact been patented.

Claims Rejections - 35 U.S.C. §112, First Paragraph

10) Claims 22-26 and 29 are rejected under 35 U.S.C. §112, first paragraph, as failing to provide an enabling disclosure, because the specification does not provide evidence that the biological materials of the claimed invention are (1) known and readily available to the public; (2) reproducible from the written description, e.g. sequenced; or (3) deposited.

It appears that an *htrB* mutant Gram-negative bacterium is required to practice the claimed method of making and using the product, mutant endotoxin, of the instant invention. As required elements, the mutant bacterium must be known and readily available to the public, or obtainable by a reproducible method set forth in the specification. It is unclear if the mutant bacterium is publicly available, or can be reproducibly isolated from nature without undue experimentation. Therefore, suitable deposits for patent purposes is suggested. The specification appears to lack complete deposit information for the *htrB* Gram-negative mutant bacterium that is specifically recited in the instant claims. Without a publicly available deposit of the bacterial mutant, one of ordinary skill in the art could not be assured of the ability to practice the invention as claimed.

If the deposit is made under the terms of the Budapest Treaty, then an affidavit or declaration by Applicants, or a statement by an attorney of record stating that the deposit has been made under the terms of the Budapest Treaty and that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent on this application and that the deposit will be replaced if viable sample cannot be dispensed by the depository, is required to satisfy the deposit requirements. See 37 CFR 1.801-37 CFR 1.809. Further, the statement should identify the deposited mutant bacterium by its depository accession number, establish that the deposited mutant bacterium is the same as that described in the specification, and establish that the deposited bacterium was in Applicants' possession at the time of filing. *In re Lundak*, 773 F2d 1216, 227 USPQ 90 (Fed. Cir. 1985).

Claims Rejections - 35 U.S.C. §112, Second Paragraph

11) Claims 23, 24 and 29 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Claim 24 is indefinite in the recitation "comprising conjugation to a carrier protein" because it is unclear what else is encompassed or comprised in this recitation. In order to more clearly define the invention, it is suggested that Applicants replace the recitation with --conjugated to a carrier protein--.

(b) the use of non-idiomatic expression in claim 29, lines 2-4, is confusing. It is suggested that Applicants change this recitation to --for use in diagnostic assays or passive immunization, the method comprising--.

(c) Claim 23 is objected to for reciting "phenol/water extraction". To be consistent with the practice in the art, it is suggested that Applicants change the recitation to –phenol-water extraction–.

Claims Rejections - 35 U.S.C. §102

12) The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
> (e) The invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

13) Claims 22, 23 and 25 are rejected under 35 U.S.C § 102(a) as being anticipated by Lee *et al.* (*J. Biol. Chem.* 270: 27151-27159, November 1995).

Lee *et al.* teach a method of making in a Gram negative bacterial pathogen, *E. coli*, a mutant endotoxin lacking one or more myristic acid substitutions in the lipid A. Thus, the *htrB* mutants of *E. coli* are taught. The LOS isolated from the *htrB* mutants did not show reactivity with the 6E4 monoclonal antibody which is specific for the wild type LOS. An *htrB* mutant of a non-typable *Haemophilus influenzae* type b is also taught (see abstract; page 27152, left column).

The mutant endotoxin is purified by proteinase K digestion (see the paragraph bridging pages 27152 and 27153). The lipid A of the mutant endotoxin showed a tetraacyl or a pentaacyl species indicative of loss of one or both of the myristic acid substitutions. The *Haemophilus htrB* mutant endotoxin has a 50% reduction in the LOS species containing two phosphoethanolamines (see page 27168, left column).

Claims 22, 23 and 25 are anticipated by Lee *et al.*

Claims Rejections - 35 U.S.C. §103(a)

14) The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person. having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 148 USPQ 459, that are applied for establishing a background for determining obviousness under 35 U.S.C. § 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or unobviousness.

15) Claims 24, 26 and 29 are rejected under 35 U.S.C. §103(a) as being unpatentable over Lee *et al.* (*J. Biol. Chem.* 270: 27151-27159, 1995) as applied to 22 above, and further in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992).

The teachings of Lee *et al.* have been explained above, which do not disclose conjugating the mutant endotoxin to a carrier protein or raising endotoxin-specific antisera as recited in the instant claims for use in diagnostic assays or in passive immunization.

Gupta *et al.* teach conjugating a deacylated endotoxin of a Gram negative bacterial pathogen to a protein carrier to produce an immunogenic conjugate vaccine that can be used to

raise endotoxin-specific antisera by administering it to an animal (see abstract, and pages 3202 and 3203).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use Lee's mutant endotoxin lacking one or more myristic acid substitutions in the lipid A to a carrier protein as taught by Gupta *et al.* for a deacylated endotoxin to produce the conjugate of the instant invention and use it in a method of raising endotoxin-specific antisera as taught by Gupta *et al.* for use in passive immunization or in diagnostic assays. One skilled in the art would have had a reasonable expectation of success in producing the conjugate and the method of raising antisera of the instant invention since the *htrB* mutant endotoxin lacking secondary acyl chains is expected to function significantly no differently than the chemically deacylated endotoxin taught by Gupta *et al.* in a conjugate. Absent evidence to the contrary, claims 24, 26 and 29, as a whole, are obvious over the prior art of record.

16) Claim 29 is rejected under 35 U.S.C. §103(a) as being unpatentable over Lee *et al.* (*J. Biol. Chem.* 270: 27151-27159, 1995) as applied to 22 above, and further in view of Sprouse *et al.* (US 5,641,492).

The reference of Sprouse *et al.* is applied in this 103 rejection because it qualifies as prior art under subsection (e) of 35 U.S.C. § 102 and accordingly is not disqualified under U.S.C. 103(a).

The teachings of Lee *et al.* have been explained above, which do not disclose raising endotoxin-specific antisera as recited in the instant claim for use in diagnostic assays or in passive immunization.

Sprouse *et al.* disclose a method of producing endotoxin-specific antisera for use in passive immunization or for diagnostic purposes by immunizing an individual with a vaccine comprising a mutant Gram negative bacterial pathogen or an endotoxin obtained from the mutant bacterium or a detoxified endotoxin (see columns 3, 4 and 7). The resultant hyperimmune sera provides protection against endotoxin-associated diseases (see column 12, lines 50-55; claim 7 and Figure 3). The hyperimmune serum is also used in a DEAE column (see column 13).

Given the prior art teachings that an *htrB* mutant bacterium produces less toxic endotoxin and that such mutant bacteria or detoxified mutant endotoxin can be used as a vaccine to raise protective antisera for use in passive immunization against Gram negative bacterial infections, it would have been obvious to one of ordinary skill in the art at the time of the instant invention to use Lee's bacterial *htrB* mutant or Lee's mutant endotoxin lacking one or more secondary acyl chains to produce endotoxin-specific antisera using Sprouse's method to produce the instant invention with a reasonable expectation of success in using it for passive immunization or for diagnostic purposes.

Claim 29 is obvious over the prior art of record.

Remarks

17) Claims 22-26 and 29 stand rejected.

18) The prior art made of record and not relied upon currently in any rejection is considered pertinent to Applicants' disclosure:

- Karow *et al.* (*J. Bacteriol.* 173: 741-750, 1991) teach isolation and characterization of *E. coli htrB* mutants.
- Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) teach *E. coli htrB* mutants producing a mutant endotoxin lacking one or more myristic acid and lauric acid (see abstract and pages 7413 and 7416).
- Karow *et al.* (*Mol. Microbiol.* 5: 2285-2292, 1991) teach the sequencing, mutational analysis and transcriptional regulation of the *E. coli htrB* gene.

19) Papers related to this application may be submitted to Group 1600, AU 1641 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1. The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242.

20) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi whose telephone number is (703) 308-9347. The Examiner can

normally be reached on Monday to Friday from 8.00 am to 4.00 pm. A message may be left on the Examiner's voice mail service.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, James Housel, can be reached on (703) 308-4027. The fax phone number for this Group is (703) 305-7939.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

April 1999

James C. Housel
JAMES C. HOUSEL 4/26/99
SUPERVISORY PATENT EXAMINER

Form 1449*	Atty. Docket No.: 875.001US2	Serial No. 09/077,572
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Applicant: Michael A. Apicella et al.	
	Filing Date: June 1, 1998	Group: ~~Unknown~~ 1641

U.S. PATENT DOCUMENTS

**Examiner Initial	Document Number	Date	Name	Class	Subclass	Filing Date If Appropriate
SD	4,912,094	03/27/1990	Myers, et al.	514	54	06/29/88
SD	4,929,604	05/29/1990	Munford, et al.	514	53	05/28/96

FOREIGN PATENT DOCUMENTS

**Examiner Initial	Document Number	Date	Country	Class	Subclass	Translation Yes | No

OTHER DOCUMENTS

(Including Author, Title, Date, Pertinent Pages, Etc.)

**Examiner Initial	
SD	Karow, M., et al., "Isolation and Characterization of the Escherichia coli htrB gene, whose product is essential for bacterial viability above 33degreesC in rich media", Journal of Bacteriology, Vol. 173, No. 2, pp. 741-750, (January 1991)
SD	Karow, M., et al., "The lethal Phenotype 'caused by null mutations in the escherichia coli htrB gene is suppressed by Mutations in the accBC Operon, Encoding two subunits of acetyl coenzyme A carboxylase", Journal of Bacteriology, Vol. 174, No. 22, pp. 7407-7418, (November 1992)
SD	Lee, N., et al., "Mutation of the htrB locus of Haemophilus influenzae nontypable strain 2019 is associated with modifications of lipid A and physophorylation of the lipo-oligosaccharide", The Journal of Biological Chemistry, Vol. 270, No. 45, pp. 27151-27159, (November 10, 1995)
SD	Lehmann, V., et al., "Isolation of a mutant from Salmonella typhimurium producing acyl-deficient lipopolysaccharides", 459-464, (1988)

Examiner	SD	Date Considered	26 Apr. 99

*Substitute Disclosure Statement Form (PTO-1449)

**EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Notice of References Cited

Application No.	Applicant(s)		
09/077,572	Apicella et al.		
Examiner		Group Art Unit	Page 1 of 1
S. Devi, Ph.D.		1641	

U.S. PATENT DOCUMENTS

		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
	A	US 5,641,492	06/24/97	Sprouse et al.	424	258.1
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
	N						
	O						
	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
	U	Gupta et al. Infect. Immun. 60: 3201-3208	1992
	V	Karow et al. Mol. Microbiol. 5: 2285-2292	1991
	W		
	X		

SD 26 Apr. 99

Form PTO 948 (Rev. 6/97) U.S. DEPARTMENT OF COMMERCE - Patent and Trademark Office Application No. 9/077,572

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

The drawing(s) filed (insert date) 10-13-98 are:

A. ☐ approved by the Draftsperson under 37 CFR 1.84 or 1.152.

B. ☒ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 for the reasons indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawing must be sumitted according to the instructions on the back of this notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings: Black ink. Color.
   - ___ Color drawings are not acceptable until petiton is granted. Fig(s) ___
   - ___ Pencil and non black ink not permitted. Fig(s) ___
2. PHOTOGRAPHS. 37 CFR 1.84 (b)
   - ___ 1 full-tone set is required. Fig(s) ___
   - ☒ Photographs not properly mounted (must use brystol board or photographic double-weight paper). Fig(s) 4
   - ☒ Poor quality (half-tone). Fig(s) 4
3. TYPE OF PAPER. 37 CFR 1.84(e)
   - ___ Paper not flexible, strong, white, and durable. Fig(s) ___
   - ___ Erasures, alterations, overwritings, interlineations, folds, copy machine marks not accepted. Fig(s) ___
   - ___ Mylar, velum paper is not acceptable (too thin). Fig(s) ___
4. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:
   - ___ 21.0 cm by 29.7 cm (DIN size A4)
   - ___ 21.6 cm by 27.9 cm (8 1/2 x 11 inches)
   - ___ All drawing sheets not the same size. Sheet(s) ___
   - ___ Drawings sheets not an acceptable size. Fig(s) ___
5. MARGINS. 37 CFR 1.84(g): Acceptable margins:

   Top 2.5 cm Left 2.5cm Right 1.5 cm Bottom 1.0 cm
   SIZE: A4 Size

   Top 2.5 cm Left 2.5 cm Right 1.5 cm Bottom 1.0 cm
   SIZE: 8 1/2 x 11

   Margins not acceptable. Fig(s) 1 4 7
   - ___ Top (T) ☒ Left (L)
   - ___ Right (R) ___ Bottom (B)
6. VIEWS. 37 CFR 1.84(h)
   REMINDER: Specification may require revision to correspond to drawing changes.
   Partial views. 37 CFR 1.84(h)(2)
   - ___ Brackets needed to show figure as one entity. Fig(s) ___
   - ___ Views not labeled separately or properly. Fig(s) ___
   - ___ Enlarged view not labeled separetely or properly. Fig(s) ___
7. SECTIONAL VIEWS. 37 CFR 1.84 (h)(3)
   - ___ Hatching not indicated for sectional portions of an object. Fig(s) ___
   - ___ Sectional designation should be noted with Arabic or Roman numbers. Fig(s) ___
8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)
   - ___ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) ___
9. SCALE. 37 CFR 1.84(k)
   - ___ Scale not large enough to show mechanism without crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) ___
10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(l)
    - ☒ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (poor line quality). Fig(s) 1-7
11. SHADING. 37 CFR 1.84(m)
    - ___ Solid black areas pale. Fig(s) ___
    - ___ Solid black shading not permitted. Fig(s) ___
    - ___ Shade lines, pale, rough and blurred. Fig(s) ___
12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)
    - ☒ Numbers and reference characters not plain and legible. Fig(s) 1-7
    - ___ Figure legends are poor. Fig(s) ___
    - ___ Numbers and reference characters not oriented in the same direction as the view. 37 CFR 1.84(p)(1) Fig(s) ___
    - ___ English alphabet not used. 37 CFR 1.84(p)(2) Figs ___
    - ☒ Numbers, letters and reference characters must be at least .32 cm (1/8 inch) in height. 37 CFR 1.84(p)(3) Fig(s) 5-7
13. LEAD LINES. 37 CFR 1.84(q)
    - ___ Lead lines cross each other. Fig(s) ___
    - ___ Lead lines missing. Fig(s) ___
14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(t)
    - ___ Sheets not numbered consecutively, and in Arabic numerals beginning with number 1. Sheet(s) ___
15. NUMBERING OF VIEWS. 37 CFR 1.84(u)
    - ___ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) ___
16. CORRECTIONS. 37 CFR 1.84(w)
    - ___ Corrections not made from prior PTO-948 dated ___
17. DESIGN DRAWINGS. 37 CFR 1.152
    - ___ Surface shading shown not appropriate. Fig(s) ___
    - ___ Solid black shading not used for color contrast. Fig(s) ___

COMMENTS

REVIEWER [illegible] DATE 11-22-99 TELEPHONE NO. 703 305 8430

ATTACHMENT TO PAPER NO. 11

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al.

Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Docket No.: 875.001US2
Filed: October 13, 1998
Examiner: S. Devi

Serial No.: 09/077,572
Due Date: August 28, 1999 (Saturday)
Group Art Unit: 1641

Assistant Commissioner for Patents
Washington, D.C. 20231

We are transmitting herewith the following attached items (as indicated with an "X"):

X A return postcard.
X An Amendment and Response (7 Pages).
X Petition for Extension of Time (1 pg.) (Fee of $110.00 to be charged to Deposit Acct. No. 19-0743.)
X A Supplemental Information Disclosure Statement (1 pgs.), Form 1449 (1 pgs.). References NOT enclosed, cited in parent application. (Fee of $240.00 to be charged to Deposit Acct. No. 19-0743.)
X Declaration Under 37 C.F.R. 1.132 (8 pgs.).

Please consider this a PETITION FOR EXTENSION OF TIME for sufficient number of months to enter these papers and please charge any additional required fees or credit overpayment to Deposit Account No. 19-0743.

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this Transmittal Letter and the paper, as described above, are being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 30th day of August, 1999 (Monday).

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)

By: _____
Atty: Ann S. Viksnins
Reg. No. 37,748

Customer Number 21186

(GENERAL)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi	
Serial No.:	09/077,572	Group Art Unit: 1641	
Filed:	October 13, 1998	Docket: 875.001US2	
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA		

PETITION FOR A ONE-MONTH EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

In accordance with the provisions of 37 C.F.R. § 1.136(a), it is respectfully requested that a one-month extension of time be granted in which to respond to the Office Action mailed April 28, 1999, said period of response being extended from July 28, 1999 to August 28, 1999 (Saturday).

Please charge the required fee of $110.00 and any additional fees to Deposit Account No. 19-0743.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 30 August 1999 By _____
Ann S. Viksnins
Reg. No. 37,748

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 30th day of August, 1999 (Monday).

Ann S Viksnins
Name

_____
Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi	
Serial No.:	09/077,572	Group Art Unit: 1641	
Filed:	June 1, 1998	Docket: 875.001US2	
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA		

AMENDMENT AND RESPONSE

Assistant Commissioner for Patents
Washington, D.C. 20231

In response to the Office Action mailed April 28, 1999, please amend the application as follows.

This response is accompanied by a Petition, as well as the appropriate fee, to obtain a one-month extension of the period for responding to the Office Action, thereby moving the deadline for response from July 28, 1999 to August 28, 1999 (Saturday).

IN THE SPECIFICATION

On page 1, in the first line, after the title, please insert the following:

--This application is a national stage filing under 35 U.S.C. § 371 of international patent application number PCT/US96/18984, filed on November 27, 1996, which in turn is an international filing of U.S. patent application number 08/565,943 filed on December 1, 1995.--

On page 12, line 13, please delete "acylxyacyl" and substitute therefor --acyloxyacyl--.

IN THE CLAIMS

Please amend the following claims 22-26 and 29, and add new claims 30 and 31:

22. (Amended) A method of making [in a gram-negative bacterial pathogen] a mutant endotoxin [of substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen, the method] comprising mutating an *htrB* gene within [the] a gram-negative bacterial pathogen, wherein [said mutation causes a phenotype of a resultant *htrB* mutant characterized by a mutant] the *htrB* gene encodes an endotoxin lacking one or more secondary acyl chains of lipid A contained in the wild type gram-negative bacterial pathogen.

and wherein the mutant endotoxin has substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen.

23. (Amended) A mutant endotoxin [of substantially reduced toxicity,] made according to the method of claim 22, wherein the mutant endotoxin [having substantially reduced toxicity] was purified from the *htrB* mutant by [a process selected from the group consisting of a phenol/water] phenol-water extraction[, and a] or by protease digestion[; and wherein the purified mutant endotoxin having substantially reduced toxicity is used to generate endotoxin-specific antibodies].

24. (Amended) The mutant endotoxin according to claim 23, [further comprising conjugation] wherein the mutant endotoxin is conjugated to a carrier protein.

25. (Amended) A mutant endotoxin [of substantially reduced toxicity,] made according to the method of claim 22.

26. (Amended) The mutant endotoxin according to claim 25, [further comprising conjugation] wherein the mutant endotoxin is conjugated to a carrier protein.

29. (Amended) A method for producing endotoxin-specific antisera for [a use selected from the group consisting of in diagnostic assays, and for passive immunization] use in diagnostic assays, the method [comprises] comprising

(a) immunizing an individual with a vaccine formulation comprising as an active ingredient [selected from the group consisting of] an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of [said] the gram-negative bacterial pathogen, and endotoxin isolated from the *htrB* mutant of [said] the gram-negative bacterial pathogen [said endotoxin] wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from [said] the immunized individual;

wherein [said] the *htrB* mutant lacks one or more secondary acyl chains of lipid A contained in the wild type gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.

30. (New) A method for producing endotoxin-specific antisera for use in passive immunization, the method comprising

(a) immunizing an individual with a vaccine formulation comprising an active ingredient an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, and endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the *htrB* mutant lacks one or more secondary acyl chains of lipid A contained in the wild type gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.

31. (New) The mutant endotoxin according to claim 23, wherein the purified mutant endotoxin is used to generate endotoxin-specific antibodies.

REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein, is respectfully requested.

A. Status of Claims

Reconsideration of this application as amended is requested. Claims 1-21, 27 and 28 having been cancelled in response to the Restriction Requirement; claims 22-26 and 29 having been amended; and claims 30 and 31 having been newly added; claims 22-26 and 29-31 are pending. No new subject matter has been added.

The amendments to the claims are fully supported by the specification as originally filed. The amendments are made to clarify the claims by correcting grammatical errors and eliminating redundancies, and are not intended to limit the scope of equivalents to which any claim element may be entitled.

Support for new claim 30 is found in originally filed claim 29. Support for new claim 31 is found in originally filed claim 23.

B. Drawings

Formal drawings, satisfying the objections raised by the Reviewing Draftsperson, will be submitted to the Patent Office upon notification of allowance of the claims.

C. Specification/Informalities

The Examiner objected to the present specification as it does not disclose the priority status. The specification has been amended to recite the priority information.

The Examiner stated that the recitation "acylxyacyl" hydrolase is not understood on page 12, line 13. The specification has been amended to recite "acyloxyacyl".

D. Non-Statutory Double Patenting Rejection

The Examiner rejected claims 22, 23, 25 and 29 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 19, 20 and 22 of U.S. Patent Application No. 08/565,943. Applicants will consider filing a terminal disclaimer upon notification of otherwise allowable subject matter. A terminal disclaimer may not be appropriate once the scope of allowable claims is determined in the present application.

E. 35 U.S.C. § 112, First Paragraph Rejection

The Examiner rejected claims 22-26 and 29 under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure, because the specification does not provide evidence that the biological materials of the claimed invention are (1) known and readily available to the public; (2) reproducible from the written description , *e.g.*, sequenced; or (3) deposited. This rejection is respectfully traversed.

On pages 12-15 of the specification (Example 1), Applicants disclose several methods of achieving a knockout mutation, that is, one which results in a lack of functional htrB protein as set forth in the pending claims. Applicant asserts that any of these methods can achieve a mutation in the htrB gene that will result in a lack of functional htrB enzyme and cause a phenotype characterized by endotoxin of substantially reduced toxicity. Since Applicants chose to use plasmids pB28 and pB29, however, they are willing to provide those plasmids to the public as representative embodiments. Upon receiving indication of allowable subject matter, Applicants will deposit plasmids pB28 and pB29 in compliance with the requirements set forth in *In re Lundak,* 773 F.2d 1216, 227 U.S.P.Q. 90 (Fed. Cir. 1985) and 37 C.F.R. 1.801-1.809.

F. 35 U.S.C. § 112, Second Paragraph Rejection

The Examiner rejected claims 23, 24 and 29 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention.

The Examiner states that claim 24 is indefinite in the recitation "comprising conjugation to a carrier protein"; that the use of non-idiomatic expression claim 29 is confusing; and that the term "phenol/water extraction" is not consistent with the practice in the art. Applicants have amended claims 23, 24 and 29 as suggested by the Examiner.

G. 35 U.S.C. § 102 Rejection

The Examiner rejected claims 22, 23 and 25 under 35 U.S.C. § 102(a) as being anticipated by Lee *et al.* (*J. Biol. Chem.* 270:27151-27159, November 1995).

Reconsideration of the rejection of these claims as being anticipated by Lee *et al.* is respectfully requested. As evidenced by the Declaration under 37 C.F.R. § 1.132 submitted herewith, the cited Lee *et al.* reference is not prior art, as defined by 35 U.S.C. §102(a). It is a reference of Applicants' own work describing mutation of the *htrB* locus. This reference was published less than one year from the priority date of the present application (December 1, 1995). The court in *In re Katz* stated that the fact of co-authorship, without more, does not raise a presumption that the inventorship determination on a later-filed patent application is incorrect.

687 F.2d 450, 215 U.S.P.Q. 14 (C.C.P.A. 1982). Thus, withdrawal of this rejection is appropriate and is respectfully requested.

H. 35 U.S.C. § 103 Rejections

The Examiner rejected claims 24, 26 and 29 under 35 U.S.C. § 103(a) as being unpatentable over Lee *et al.* as applied to claim 22 above, and further in view of Gupta *et al.* (*Infect. Immun.* 60:3201-3208, 1992).

Applicant respectfully submits that the Examiner has not established the *prima facie* obviousness of the present claims. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed invention. Second, the art must provide a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations. *In re Ochiai*, 37 U.S.P.Q.2d 1127 (Fed. Cir. 1997) (When evaluating the scope of a claim, every limitation in the claim must be considered.).

As discussed above, Lee *et al.* is not prior art, as defined by 35 U.S.C. §102(a). Gupta *et al.* alone does not teach or disclose all the features of the present invention. Gupta *et al.* disclose the conjugation of chemically-modified LPS to cholera toxin and other proteins. They do not, however, teach or suggest a *htrB* mutant endotoxin of substantially reduced toxicity as recited by the pending claims.

The Examiner rejected claim 29 under 35 U.S.C. § 103(a) as being unpatentable over Lee *et al.* as applied to claim 22 above, and further in view of Sprouse *et al.* (U.S. 5,641,492). Again, Lee *et al.* is not prior art, as defined by 35 U.S.C. §102(a). Sprouse *et al.* alone does not teach or disclose all the features of the present invention. Sprouse *et al.* disclose a method of making a detoxified endotoxin. Like Gupta *et al.*, however, Sprouse *et al.* do not teach or suggest a *htrB* mutant endotoxin of substantially reduced toxicity as recited by the present claims.

Applicants respectfully request that the rejections of pending claims 24, 26 and 29 under 35 U.S.C. § 103(a) be withdrawn.

S/N 09/077,572 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi	
Serial No.:	09/077,572	Group Art Unit: 1641	
Filed:	June 1, 1998	Docket: 875.001US2	
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA		

DECLARATION UNDER 37 C.F.R. § 1.132

We, Michael A. Apicella, Melvin G. Sunshine, Na-Gyong Lee, Rasappa Arumugham, and Bradford W. Gibson declare the following:

1. We are the applicants of the above-identified patent application. The above-identified patent application is a national stage filing under 35 U.S.C. § 371 of international patent application number PCT/US96/18984, filed on November 27, 1996, which in turn is an international filing of U.S. patent application number 08/565,943 filed on December 1, 1995.

2. Na-Gyong Lee, Melvin G. Sunshine, Bradford W. Gibson, and Michael A. Apicella are four of the five co-authors of the paper entitled "Mutation of the *htrB* Locus of *Haemophilus influenzae* Nontypable Strain 2019 Is Associated with Modifications of Lipid A and Phosphorylation of the Lipooligosaccharide (herein referred to as "the cited publication"), which appeared on pages 27151-27159 in the November 10, 1995 volume of *The Journal of Biological Chemistry* (Vol. 270), and which is cited by the Examiner under § 102(a). The fifth co-author of the cited publication is Jeffrey J. Engstrom.

3. The priority date of the present application is December 1, 1995, based on the filing date of the priority application 08/565,943. This priority date is less than one year after the publishing date of the cited publication.

4. Michael A. Apicella, Melvin G. Sunshine, Na-Gyong Lee, Rasappa Arumugham, and Bradford W. Gibson are co-inventors of the subject matter that is disclosed and claimed in the above-identified U.S. patent application, as stated in the Combined Declaration and Power of Attorney filed as part of this application. At the time of the present invention, the other co-author of the cited publication, Jeffrey J. Engstrom, was a technician working under the direction and supervision of Bradford W. Gibson; and thus Jeffrey J. Engstrom is not an inventor of the subject matter described and claimed in the above-identified application. Jeffrey J. Engstrom was listed as a coauthor of the

cited publication in order to receive credit for his work which was performed under the direction of, and with specific instructions from, Bradford W. Gibson. The listing of the authors names, therefore, was not an assertion of inventorship of the present invention.

5. As indicated above and in the Combined Declaration and Power of Attorney filed as part of this application, Rasappa Arumugham is a co-inventor of the subject matter that is disclosed and claimed in the above-identified U.S. patent application. Rasappa Arumugham is an inventor, but not a co-author of the cited reference, because he contributed subject matter concerning conjugation that was disclosed in the patent application, but not in the cited publication.

6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: August 18, 1999 — Michael A. Apicella

Date: August 18, 1999 — Melvin G. Sunshine

Date: _____, 1999 — Na-Gyong Lee

Date: _____, 1999 — Rasappa Arumugham

Date: _____, 1999 — Bradford W. Gibson

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1641
Filed	June 1, 1998	Docket: 875.001US2
Title	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

DECLARATION UNDER 37 C.F.R. § 1.132

We, Michael A. Apicella, Melvin G. Sunshine, Na-Gyong Lee, Rasappa Arumugham, and Bradford W. Gibson declare the following:

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6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____, 1999 _____
Michael A. Apicella

Date: _____, 1999 _____
Melvin G. Sunshine

Date: August 14, 1999 _____
Na-Gyong Lee

Date: _____, 1999 _____
Rasappa Arumugham

Date: _____, 1999 _____
Bradford W. Gibson

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1641
Filed:	June 1, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

DECLARATION UNDER 37 C.F.R. § 1.132

We, Michael A. Apicella, Melvin G. Sunshine, Na-Gyong Lee, Rasappa Arumugham, and Bradford W. Gibson declare the following:

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6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____, 1999 _____
Michael A. Apicella

Date: _____, 1999 _____
Melvin G. Sunshine

Date: _____, 1999 _____
Na-Gyong Lee

Date: August 24, 1999 _____
Rasappa Arumugham

Date: _____, 1999 _____
Bradford W. Gibson

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1641
Filed:	June 1, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

DECLARATION UNDER 37 C.F.R. § 1.132

We, Michael A. Apicella, Melvin G. Sunshine, Na-Gyong Lee, Rasappa Arumugham, and Bradford W. Gibson declare the following:

1. We are the applicants of the above-identified patent application. The above-identified patent application is a national stage filing under 35 U.S.C. § 371 of international patent application number PCT/US96/18984, filed on November 27, 1996, which in turn is an international filing of U.S. patent application number 08/565,943 filed on December 1, 1995.

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4. Michael A. Apicella, Melvin G. Sunshine, Na-Gyong Lee, Rasappa Arumugham, and Bradford W. Gibson are co-inventors of the subject matter that is disclosed and claimed in the above-identified U.S. patent application, as stated in the Combined Declaration and Power of Attorney filed as part of this application. At the time of the present invention, the other co-author of the cited publication, Jeffrey J. Engstrom, was a technician working under the direction and supervision of Bradford W. Gibson; and thus Jeffrey J. Engstrom is not an inventor of the subject matter described and claimed in the above-identified application. Jeffrey J. Engstrom was listed as a coauthor of the

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6. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____, 1999 _____
Michael A. Apicella

Date: _____, 1999 _____
Melvin G. Sunshine

Date: _____, 1999 _____
Na-Gyong Lee

Date: _____, 1999 _____
Rasappa Arumugham

Date: August 25, 1999 _____
Bradford W. Gibson

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al. Examiner: S. Devi
Serial No.: 09/077,572 Group Art Unit: 1641
Filed: October 13, 1998 Docket: 875.001US2
Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

In compliance with the duty imposed by 37 C.F.R. § 1.56, and in accordance with 37 C.F.R. §§ 1.97 *et. seq.*, the enclosed materials are brought to the attention of the Examiner for review in connection with the above-identified patent application. Pursuant to the provisions of MPEP 609, Applicants request that a copy of the 1449 form, initialled as being considered by the Examiner be returned to the Applicants.

In accordance with 37 C.F.R. §1.98(d), copies of the listed documents are not provided as these references were previously cited by or submitted to the U.S. Patent Office in connection with Applicants' prior U.S. application, Serial No. 08/565,943, filed on December 1, 1995, which is relied upon for an earlier filing date under 35 U.S.C. §120.

Please charge the required fee of $240.00 to Deposit Account No. 19-0743.

Applicants respectfully request consideration of these references during prosecution of the above-identified matter. The Examiner is invited to contact the Applicants' Representative at the below-listed telephone number if there are any questions regarding this communication.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 30 August 1999 By _____
Ann S. Viksnins
Reg. No. 37,748

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents, Washington, D.C. 20231 on August 30, 1999.

Ann S. Viksnins
Name

_____
Signature

Form 1449*	Atty. Docket No.: 875.001US2	Serial No. 09/077,572
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Applicant: Michael A. Apicella et al.	
	Filing Date: October 13, 1998	Group: 1641

U.S. PATENT DOCUMENTS

**Examiner Initial	Document Number	Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

**Examiner Initial	Document Number	Country	Class	Subclass	Translation Yes	No

OTHER DOCUMENTS
(Including Author, Title, Date, Pertinent Pages, Etc.)

**Examiner Initial	
	Brooks, G.F., et al., "Enteric Gram-negative rods (enterobacteriaceae)", Medical Microbiology, p. 206, (1995)
	Karow, M.L., "Molecular Genetics of the Escherichia coli HTRB gene", (1992)

Examiner	Date Considered

*Substitute Disclosure Statement Form (PTO-1449)

**EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

UNITED S[illegible]ES DEPARTMENT OF COMMER[illegible]
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

15

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/077,572	10/13/98	APICELLA M	875001US2

HM12/0104
SCHWEGMAN LUNDBERG WOESSNER & KLUTH
PO BOX 2938
MINNEAPOLIS MN 55402

EXAMINER	
DEVI, S	
ART UNIT	**PAPER NUMBER**
1641	16

DATE MAILED: 01/04/00

OTPE JC100 JUN 11 2002 PATENT & TRADEMARK OFFICE

MAR. 4, 2000 – 2 MO.
DV APR. 4, 2000 – 3 MO.
JULY 4, 2000 – 6 MO.

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Schwegman, Lundberg,
Woessner & Kluth, P.A.

JAN 10 2000

RECEIVED

PTO-90C (Rev. 2/95)

2 - Mail Copy

Office Action Summary	Application No. 09/077,572	Applicant(s) Apicella et al.
	Examiner S. Devi, Ph.D.	Group Art Unit 1641

☒ Responsive to communication(s) filed on *Sep 3, 1999*.

☒ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *22-26 and 29-31* is/~~are~~ pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *22-26 and 29-31* is/~~are~~ rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *15*

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

DETAILED ACTION

Applicants' Amendment

1) Acknowledgment is made of Applicants' amendment filed 09/03/99 (paper no. 13) in response to the Office Action mailed 04/28/99 (paper no. 11). With this, Applicants have amended the specification.

Status of Claims

2) The non-elected claims 33-35 and elected linking claims 36, 42 and 43 have been canceled.

Claims 22-26 and 29 have been amended.

New claims 30-31 have been added.

Claims 22-26 and 29-31 are pending and are under examination.

Information Disclosure Statement

3) Acknowledgment is made of Applicants' Supplemental Information Disclosure Statement filed 09/03/99 (paper no. 15). One of the documents referred to therein has been considered and the other having incomplete citation is lined through. A signed copy of the IDS is attached to this Office Action (paper no. 16).

Declaration under 37 C.F.R § 1.132

4) Acknowledgment is made of Applicants' declaration filed 09/03/99 (paper no. 14) under 37 C.F.R § 1.132.

Prior Citation of Title 35 Sections

5) The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office Action.

Prior Citation of References

6) The references cited or used as prior art in support of one or more rejections in the instant Office Action and not included on an attached form PTO-892 or form PTO-1449 have been previously cited and made of record.

Objections Withdrawn

7) The objection to the specification made in paragraph 7(a) of the Office Action mailed 04/28/99 (paper no. 11) is withdrawn in light of Applicants' amendment to the first paragraph of the specification to reflect the correct continuity status of the instant application.

8) The objection to the specification made in paragraph 7(b) of the Office Action mailed 04/28/99 (paper no. 11) with regard to a spelling error is withdrawn in light of Applicants' amendment of the specification.

Objection Maintained

9) The objection to the drawings made in paragraph 6 of the Office Action mailed 04/28/99 (paper no. 11) is maintained for reasons set forth therein. Applicants assure the Office that corrected formal drawings will be submitted upon notification of allowance of claims.

Rejections Withdrawn

10) The rejection of claims 23, 24 and 29 made in paragraph 11 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendments to the claims.

11) The rejection of claims 22, 23 and 25 made in paragraph 13 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 102(a) as being anticipated by Lee *et al.* (*J. Biol. Chem.* 270: 27151-27159, November 1995), is withdrawn in light of Applicants' declaration filed under 37 C.F.R § 1.132. Applicants state that the fifth co-author of the Lee's publication, Jeffrey J. Engstrom, is not an inventor.

12) The rejection of claims 24, 26 and 29 made in paragraph 15 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 103(a) as being unpatentable over Lee *et al.* (*J. Biol. Chem.* 270: 27151-27159, 1995) as applied to 22 and further in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992) is withdrawn in light of Applicants' declaration filed under 37 C.F.R § 1.132 stating that the fifth co-author of the primary reference of Lee *et al.*, Jeffrey J. Engstrom, is not an inventor.

13) The rejection of claim 29 made in paragraph 15 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 103(a) as being unpatentable over Lee *et al.* (*J. Biol. Chem.*

270: 27151-27159, 1995) as applied to claim 22, and further in view of Sprouse *et al.* (US 5,641,492) is withdrawn in light of Applicants' declaration filed under 37 C.F.R § 1.132 stating that the fifth co-author of the primary reference of Lee *et al.*, Jeffrey J. Engstrom, is not an inventor.

Rejections Maintained

14) The rejection of claims 22, 23, 25 and 29 made in paragraph 9 of the Office Action mailed 04/28/99 (paper no. 11) under the judicially created provisional obviousness type double patenting is maintained for reasons set forth therein. Applicants state that if appropriate, they will consider filing a terminal disclaimer upon notification of allowable subject matter.

15) The rejection of claims 22-26 and 29 made in paragraph 10 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 112, first paragraph, with regard to the deposit of the mutant bacterium is maintained for reasons set forth therein. Applicants assure the Office that upon receiving indication of allowable subject matter, Applicants will deposit plasmids pB28 and pB29 in compliance 37 C.F.R 1.801-1.809.

New Rejections

Applicants are asked to note the new rejections made in this Office Action. The Applicants' amendment including the addition of new claims, necessitated the new ground(s) of rejection presented in this Office Action.

Rejection(s) under 35 U.S.C. § 112, First Paragraph

16) Claims 29 and 30 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Claims 29 and 30 currently encompass a method for producing endotoxin-specific antisera by immunizing an individual with a vaccine formulation comprising **three** active ingredients, i.e., 1) An *htrB* mutant of a gram-negative bacterial pathogen; 2) An endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen **and,** 3) An endotoxin isolated from the *htrB*

mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein. However, there appears to be no support in the instant specification for such a method of producing an endotoxin-specific antisera using a three-component vaccine composition. Applicants have not pointed to the specific parts of the disclosure that support this added limitation in the claims. Therefore, the limitation in the claims is considered to be new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P 608.04 to 608.04(c).

Applicants are respectfully requested to point to the descriptive support in the specification as filed, for the newly added limitation, or to remove the new matter from the claim.

Rejection(s) under 35 U.S.C. § 112, Second Paragraph

17) Claims 22-26 and 29-31 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Claim 22 lacks antecedence for the recitation "**the wild type** gram-negative bacterial pathogen" (see lines 6 and 8) (Emphasis added), because the earlier occurrence of this recitation has been canceled via the amendment filed 09/03/99.

(b) Claims 29 and 30 lack antecedence for the recitation "**the wild type** gram-negative bacterial pathogen" (see part b).

(c) Claim 23 lacks proper antecedence for the recitation "**the** *htrB* mutant" (see line 3) (Emphasis added). Claim 23 depends from claim 22, which recites a "gram-negative bacterial pathogen" comprising a mutated *htrB* gene, but not a "*htrB* mutant".

(d) Claim 29 is vague, confusing and/or incorrect in reciting "immunizing an individual with a vaccine formulation comprising as an active ingredient an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, **and** endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen

wherein the endotoxin is conjugated to a carrier protein" (see part a of the claim) (Emphasis added). Note that the Markush language "selected from the group consisting of" (see line 5) has been removed via the amendment filed 09/03/99. As the claim is drafted currently, the vaccine formulation used to immunize an individual comprises three active ingredients: the mutant bacterium plus endotoxin of the mutant bacterium plus such endotoxin conjugated to a carrier protein. However, such a method of producing endotoxin-specific antisera by immunizing an individual with a vaccine formulation comprising **three** active ingredients is not supported by the instant specification.

(e) Analogous criticism as explained above in paragraph (d) applies to claim 30.

(f) In claim 31, it is unclear how the step of using the mutant endotoxin to generate antibodies further limits the mutant endotoxin itself. Clarification is requested.

(g) It is not clear what the differences are, if any, between the methods of claims 29 and 30, both of which contain identical steps.

(h) Claim 22 is confusing and/or incomplete because it is unclear how just "mutating an *htrB* gene within a gram negative bacterial pathogen" can lead to a "method of making a mutant endotoxin". The process of "mutating an *htrB* gene within a gram negative bacterial pathogen" would result in a mutated bacterium, not in a mutant endotoxin. Clarification is required.

Rejection under 35 U.S.C. § 102(b)

18) The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejection under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

19) Claim 22 is rejected under 35 U.S.C § 102(b) as being anticipated by Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992, already of record).

Karow *et al.* teach a method of making a mutant endotoxin or LPS from a Gram negative bacterial pathogen, *E. coli,* containing a mutated *htrB* gene. The mutant bacterium produces a mutant endotoxin lacking one or more lauric acid and myristic acid (i.e., secondary acyl chains of

lipid A) (see abstract; page 7413 left column; paragraph bridging left and right columns on page 7416, and page 7409, left column, under 'Fatty acid analysis'). The description provided in the Figure 4 legend indicates that the *htrB* mutant endotoxin is isolated from the *htrB* mutant bacterium (see page 7413). That the absence of one or more lauric acid and myristic acid in the lipid A renders the bacterial LPS substantially less toxic is inherent from the teachings of Karow *et al.*

Claim 22 is anticipated by Karow *et al.*

Rejection(s) under 35 U.S.C. § 103(a)

20) Claims 23-26 and 31 are rejected under 35 U.S.C. §103(a) as being unpatentable over Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992, already of record) as applied to claim 22 above, and further in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992, already of record).

The teaching of Karow *et al.* is explained above which does not expressly disclose a method of purifying the mutant endotoxin by phenol-water extraction, or conjugating the mutant endotoxin to a carrier protein, or raising antisera to the mutant endotoxin in an individual.

However, the method of purifying an endotoxin, for example, by phenol-water extraction is conventional and is well known in the art for decades. See the section 'State of the Art' below.

Similarly, conjugating a substantially less toxic endotoxin of a gram negative bacterial pathogen to a carrier protein to enhance the immunogenicity of the endotoxin is widely practiced in the art. For instance, Gupta *et al.* teach conjugating a deacylated endotoxin of a Gram negative bacterial pathogen to a protein carrier to produce an immunogenic conjugate vaccine that can be used to raise endotoxin-specific antisera by administering it to an individual animal (see abstract, and pages 3202 and 3203).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to purify Karow's mutant endotoxin lacking one or more myristic acid substitutions in the lipid A using the conventional art-known phenol-water extraction and conjugate the resultant purified mutant endotoxin to a carrier protein to raise endotoxin-specific antisera as taught by Gupta *et al.* One skilled in the art would have had a reasonable expectation of success in producing the purified mutant endotoxin and the conjugate for use as a vaccine formulation, or as

an immunogen to raise endotoxin-specific antisera of the instant invention, since the *htrB* mutant endotoxin lacking secondary acyl chains is expected to function significantly no differently in a conjugate than the deacylated endotoxin taught by Gupta *et al.* Absent evidence to the contrary, claims 23-26 and 31, as a whole, are obvious over the prior art of record.

Objection

21) Claim 30 is grammatically incorrect in the recitation "comprising an active ingredient an htrB mutant" (see lines 3 and 4).

Remarks

22) Claims 22-26 and 29-31 stand rejected.

23) The prior art made of record and not relied upon currently in any rejection is considered pertinent to Applicants' disclosure:

- Westphal *et al.* (*Methods Carbohydr. Chem.* 5: 83-91, 1965) teach phenol water extraction of gram negative bacterial lipopolysaccharides (see entire document).
- Karow ML. Molecular Genetics of the *Escherichia coli htrB* gene. Ph.D. Dissertation, The University of Utah, 1992.
- McLaughlin *et al.* (*J. Bacteriol.* 174: 6455-6459, 1992) teach a method of preparing an endotoxin derived from a gram negative bacterial mutant using microphenol method and proteinase K treatment (see page 6456, left column).

24) THIS ACTION IS MADE FINAL. Applicants are reminded of the extension of time policy as set forth in 37 C.F.R 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date

of this final action.

25) Papers related to this application may be submitted to Group 1600, AU 1641 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1 (CM1). The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242.

26) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. The Examiner can normally be reached on Monday to Friday from 8.00 a.m to 4.00 p.m.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, James Housel, can be reached on (703) 308-4027.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

January 2000

James C. Housel
JAMES C. HOUSEL 1/3/00
SUPERVISORY PATENT EXAMINER

Form 1449*	Atty. Docket No.: 875.00TUS2	Serial No. 09/077,572
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	Applicant: Michael A. Apicella et al.	
	Filing Date: October 13, 1998	Group: 1641

U.S. PATENT DOCUMENTS

**Examiner Initial	Document Number	Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS

**Examiner Initial	Document Number	Date	Country	Class	Subclass	Translation Yes	No

OTHER DOCUMENTS
(Including Author, Title, Date, Pertinent Pages, Etc.)

**Examiner Initial	
SD	Brooks, G.F., et al., "Enteric Gram-negative rods (enterobacteriaceae)", Medical Microbiology, p. 206, (1995)
	~~Karow, M.L., "Molecular Genetics of the Escherichia coli HTRB gene", (1992)~~

Examiner SD	Date Considered 20 Dec. 99

*Substitute Disclosure Statement Form (PTO-1449)

**EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Notice of References Cited

Application No.	Applicant(s)		
09/077,572	Apicella et al.		
Examiner	Group Art Unit	Page 1 of 1	
S. Devi, Ph.D.	1641		

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS
	A					
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUBCLASS
	N						
	O						
	P						
	Q						
	R						
	S						
	T						

NON-PATENT DOCUMENTS

*		DOCUMENT (Including Author, Title, Source, and Pertinent Pages)	DATE
	U	Westphal et al. Methods in Carbohydrate Chemistry 5: 83-91	1965
	V	McLaughlin et al. J. Bacteriol. 174: 6455-6459	1992
X	W	Karow ML. Molecular Genetics of the Escherichia coli htrB gene. Ph.D. Dissertation, The Utah University	1992
	X		

* A copy of this reference is not being furnished with this Office action.
(See Manual of Patent Examining Procedure, Section 707.05(a).)

In re Patent Application of: Michael A. Apicella et al.
Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA
Serial No.: 09/077,572 Filing Date: October 13, 1998

Receipt is hereby acknowledged for the following in the United States Patent and Trademark Office:

CONTENTS: An Amendment and Response (8 Pages); Declaration of Drs. Gibson and Apicella Under 37 C.F.R. 1.132 (3 pgs.); Petition for Extension of Time (1 pg.); a check in the amount of $870.00 to cover the Extension of Time Fee; Notice of Appeal (1 pg.); a check in the amount of $300.00 to cover the Notice of Appeal filing fee; a Return Postcard and TRANSMITTAL SHEET.

Mailed: June 30, 2000
ASV/cbb

Docket No.: 875.001US2
Due Date: July 4, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al.

Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Docket No.: 875.001US2
Filed: October 13, 1998
Examiner: S. Devi

Serial No.: 09/077,572
Due Date: July 4, 2000
Group Art Unit: 1641

BOX AF
Commissioner for Patents
Washington, D.C. 20231

We are transmitting herewith the following attached items (as indicated with an "X"):

X A return postcard.
X An Amendment and Response (8 Pages).
X Declaration of Drs. Gibson and Apicella Under 37 C.F.R. 1.132 (3 pgs.).
X Petition for Extension of Time (1 pg.)
X A check in the amount of $870.00 to cover the Extension of Time Fee.
X Notice of Appeal (1 pg.).
X A check in the amount of $300.00 to cover the Notice of Appeal filing fee.

Please consider this a PETITION FOR EXTENSION OF TIME for sufficient number of months to enter these papers and please charge any additional required fees or credit overpayment to Deposit Account No. 19-0743.

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this Transmittal Letter and the paper, as described above, are being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: BOX AF, Commissioner for Patents, Washington, D.C. 20231, on this 30th day of June, 2000.

By: _____

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)

Atty: Ann S. Viksnins
Reg. No. 37,748

Customer Number 21186

(GENERAL)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi	
Serial No.:	09/077,572	Group Art Unit: 1641	
Filed:	October 13, 1998	Docket: 875.001US2	
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA		

NOTICE OF APPEAL FROM THE DECISION OF THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

BOX AF
Commissioner for Patents
Washington, D.C. 20231

In compliance with 37 C.F.R. § 1.191, Applicants hereby appeal to the Board of Patent Appeals and Interferences from the decision dated January 4, 2000, of the Examiner rejecting claims 22-26 and 29-31 of the above-identified patent application.

A request for an extension of time to respond to the Examiner's rejection is submitted herewith along with payment of the required extension fee.

Our check in the amount of $300.00 is enclosed to pay the Notice of Appeal fee under 37 C.F.R. § 1.17(b). Please charge any required additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By Applicants' Attorneys,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 30 Jun 2000 By _____
Ann S. Viksnins
Reg. No. 37,748

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to BOX AF, Commissioner of Patents, Washington, D.C. 20231 on June 30, 2000.

Ann S. Viksnins
Name

_____
Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1641
Filed:	October 13, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

PETITION FOR A THREE-MONTH EXTENSION OF TIME

BOX AF
Commissioner for Patents
Washington, D.C. 20231

In accordance with the provisions of 37 C.F.R. § 1.136(a), it is respectfully requested that a three-month extension of time be granted in which to respond to the final Office Action mailed January 4, 2000, said period of response being extended from April 4, 2000 to July 4, 2000.

Our check in the amount of $870.00 is enclosed to cover the required extension fee. Please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 30 June 2000 By _____
Ann S. Viksnins
Reg. No. 37,748

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: BOX AF, Commissioner for Patents, Washington, D.C. 20231, on this 30th day of June, 2000.

Ann S. Viksnins
Name

_____
Signature

EXPEDITED PROCEDURE - EXAMINING GROUP 1641

S/N 09/077,572 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1641
Filed:	October 13, 1998	Docket: 875.0ō1US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116

Box AF
Assistant Commissioner for Patents
Washington, D.C. 20231

In response to the final Office Action mailed January 4, 2000, please amend the application as indicated below.

This response is accompanied by a Petition, as well as the appropriate fee, to obtain a three-month extension of the period for responding to the Office Action, thereby moving the deadline for response from April 4, 2000 to July 4, 2000.

In the Claims

Please cancel claims 30 and 31 without prejudice..

Please amend claims 22, 23 and 29 as follows. (For the Examiner's convenience, non-amended dependent claims 24-26 are also included).

22. (Amended) A method of making a mutant endotoxin comprising

mutating an *htrB* gene within a gram-negative bacterial pathogen to form an *htrB* mutant pathogen, wherein the *htrB* gene encodes an endotoxin lacking one or more secondary acyl chains of lipid A contained in [the] a wild type gram-negative bacterial pathogen, and wherein the mutant endotoxin has substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen, and

purifying the mutant endotoxin from the *htrB* mutant pathogen.

23. (Amended) A mutant endotoxin made according to the method of claim 22, wherein the mutant endotoxin was purified from the *htrB* mutant pathogen by phenol-water extraction or by protease digestion.

24. The mutant endotoxin according to claim 23, wherein the mutant endotoxin is conjugated to a carrier protein.

25. A mutant endotoxin made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, wherein the mutant endotoxin is conjugated to a carrier protein.

29. (Amended) A method for producing endotoxin-specific antisera [for use in diagnostic assays], the method comprising

(a) immunizing an individual with a vaccine formulation comprising [as an active ingredient] an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, [and] or endotoxin [isolated] purified from the *htrB* mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the *htrB* mutant lacks one or more secondary acyl chains of lipid A contained in [the] a wild type gram-negative bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.

Please add the following new claims 44 and 45:

44. (New) The method of claim 22 wherein the gram-negative bacterial pathogen is of the genera Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella or Pseudomonas.

45. (New) The method of claim 29 wherein the gram-negative bacterial pathogen is of the genera Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella or Pseudomonas.

REMARKS

Applicant has carefully reviewed and considered the Office Action mailed on January 4, 2000, and the reference cited therewith.

Claims 22-23, and 29 are amended, claims 44 and 45 are newly added, and claims 30 and 31 are canceled; as a result, claims 22-26, 29, 44 and 45 are now pending in this application. No new subject matter has been added to the claims. The amendments to the claims are fully supported by the specification as originally filed. The amendments are made to clarify the claims, and are not intended to limit the scope of equivalents to which any claim element may be entitled.

A. Drawings

Formal drawings, satisfying the objections raised by the Reviewing Draftsperson, will be submitted to the Patent Office upon notification of allowance of the claims.

B. Non-Statutory Double Patenting Rejection

The Examiner rejected claims 22, 23, 25 and 29 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 19, 20 and 22 of U.S. Patent Application No. 08/565,943. Applicants will consider filing a terminal disclaimer upon notification of otherwise allowable subject matter. A terminal disclaimer may not be appropriate once the scope of allowable claims is determined in the present application.

C. 35 U.S.C. § 112, First Paragraph Rejection

The Examiner rejected claims 22-26 and 29 under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure, because the specification does not provide evidence that the biological materials of the claimed invention are (1) known and readily available to the public; (2) reproducible from the written description, *e.g.*, sequenced; or (3) deposited. This rejection is respectfully traversed.

Upon receiving indication of allowable subject matter, Applicants will deposit plasmids pB28 and pB29 in compliance with the requirements set forth 37 C.F.R. 1.801-1.809.

The Examiner rejected claims 29 and 30 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Claim 29 has been amended to indicate that the listed items are in the alternative; *i.e.*, the word "and" has been replace with the word "or." Claim 30 has been canceled.

D. 35 U.S.C. § 112, Second Paragraph Rejection

The Examiner rejected claims 22-26 and 29-31 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention.

The Examiner stated that claims 22 and 29 lack antecedence for the recitation "the" wild type gram-negative bacterial pathogen. Claims 22, 29 and 30 have been amended to recite "a" wild type gram-negative bacterial pathogen. Claim 30 has been canceled.

The Examiner stated that claim 23 lacks antecedence for "the *htrB* mutant". Claim 22 has been amended to provide proper antecedence for this term.

The Examiner stated that claims 29 and 30 are vague, confusing and/or incorrect in reciting three active ingredients in the vaccine formulation. Claim 29 has been amended to recite the active ingredients in the alternative. Claim 30 has been canceled.

The Examiner stated that claim 31 is unclear. Claim 31 has been canceled.

The Examiner stated that it is not clear what the differences are between claims 29 and 30. Claim 30 has been canceled, thereby rendering this rejection moot.

E. 35 U.S.C. § 102 Rejection

The Examiner rejected claim 22 under 35 U.S.C. § 102(b) as being anticipated by Karow et al. (J. Bacteriol. 174: 7407-7418, 1992). This rejection is respectfully traversed.

A proper rejection under §102(b) requires that the cited reference identically describe or disclose all of the elements of the claimed invention. The claims as amended recite a method of

making a mutant endotoxin lacking one or more secondary acyl chains of lipid A as compared to the wild type.

The present *htrb* mutant pathogen, such as in *H. influenzae*, makes a simple truncated penta- and tetraacylated lipid A, whose structure can be derived directly from the deletion of one or two O-linked myristoyl fatty acids (C_{14}) from the parental lipid A structure. § 132 Declaration of Drs. Gibson and Apicella, ¶ 4 (hereinafter "§ 132 Declaration", attached herewith). For example, in wild type strains, *Neisseria gonorrhoeae* lipid A is hexaacylated and contains two C-12 fatty acids (lauric acid), one on each of the two glucosamines. § 132 Declaration, ¶ 5. In contrast, the *htrB* mutation in *N. gonorrhoeae* strain 1291 results in the complete deletion of one of these two lauric acid moieties to form a pentaacyl lipid A structure. *Id.* No fully hexaacylated lipid A species is seen, nor higher mass structures or new fatty acids. *Id.* The outcome for *htrB* in *N. gonorrhoeae* is similar to the *htrB* knockout in *H. influenzae*, which produced a truncated pentaacyl and tetraacyl lipid A species. *Id.*

In addition, some changes in the phosphorylation pattern in the LOS and lipid A moiety are observed between wild type and *htrB*- mutant in *N. gonorrhoeae* strain 1291. § 132 Declaration, ¶ 6. These changes involve an increased level of phosphoethanolamine (PEA) in both the lipid A moiety as well as the oligosaccharide. *Id.*

The lipid A structures created by Karow *et al.* are quite different from those of the present invention. The present inventors obtained a culture of the *E. coli htrB* mutant (hereinafter "the Karow strain" or "the Karow mutant") from Costa Georgopoulos, one of the co-authors of the article Karow *et al*, J. Bacteriol. 174:7407-7418 (1992). §132 Declaration, ¶ 7. The present inventors then performed a mass spectrometric examination of the Karow strain. *Id.* The results of this examination clearly show that the Karow organism has a set of lipid A structures different in two very important ways from the *htrb* mutant pathogens of the present invention. *Id.*

First, the Karow mutant makes a fully hexaacylated lipid A structure that is distinct in mass from the lipid A made by the parental wild-type strain. § 132 Declaration, ¶ 8. Specifically, the Karow mutant appears to contain a mixture of new unsaturated fatty acids, most likely palmitoleic (C16:1) in place of the single lauric acid (C12:0) fatty acid. *Id.* This substitution causes a shift up in mass of 26 and 54 Da from the major wild type lipid A

(molecular weight = 1798), producing new hexaacylated lipid A molecules with molecular weights of 1824 (+26, or C_2H_2) and 1852 (+ 54, or C_4H_6). *Id.*

Second, even though pentaacyl and tetraacyl substituted lipid A species are also seen in Karow's *E. coli*, these structures, when present, are not simple deletions of one and two fatty acids from the wild type (as is the case for *H. influenzae htrB*), but rather contain at least one new fatty acid not present in the small amounts of corresponding pentaacyl lipid A (MW = 1588, wild type pentaacyl lipid A) seen in the wild type lipid A preparation. § 132 Declaration, ¶ 9. The molecular weights of these two lipid A molecules are 1616 and 1406, and are consistent with a loss of the palmitoleic group (-236 Da, MW 1852--> 1616, mutant pentaacyl lipid A) and then a myristic acid group (-210 Da, MW 1616--> 1406, mutant tetraacyl lipid A). *Id.*

Thus, significant differences exist in the lipid A structures in the *htrB* gene deletion mutants of the present invention as compared to Karow's strain. In particular, Karow's strain makes both a fully acylated lipid A as well as non-fully acylated lipid A, whereas the present invention contains only penta- and tetraacylated lipid A (*i.e.*, no fully acylated lipid A molecules). Therefore, the present invention is novel over Karow *et al.*

E. 35 U.S.C. § 103 Rejection

The Examiner rejected claims 23-26 and 31 under 35 U.S.C. § 103(a) as being unpatentable over Karow et al. (J. Bacteriol. 174: 7407-7418, 1992) as applied to claim 22 above, and further in view of Gupta *et al.* (Infect. Immun. 60: 3201-3208, 1992).

Applicant respectfully submits that the Examiner has not established the *prima facie* obviousness of the present claims. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed invention. *In re Fine*, 837 F.2d 1071, 1074 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). Second, the art must provide a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 493, 20 U.S.P.Q.2d 14838, 1442 (Fed. Cir. 1991). Finally, the prior art reference must teach or suggest all of the claim limitations, and the teachings or suggestion, as well as the expectation of success, must come from the prior art, not applicant's disclosure. *Id.*

The motivation to modify the prior art must flow from some teaching in the art that suggests the desirability or incentive to make the modification needed to arrive at the claimed invention. *In re Laskowski*, 871 F.2d 115, 117, 10 U.S.P.Q.2d 1397, 1398 (Fed. Cir. 1989) ("[t]he mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification"). Karow *et al.* would not motivate the art worker to attempt to produce the claimed invention. Karow *et al.*, as discussed above, teaches the production of insertion mutations in the *E. coli htrB* gene that results in a strain that makes both a fully acylated lipid A (*i.e.*, hexaacylated lipid A) as well as non-fully acylated lipid A. In contrast, the present claims recite a pathogen that makes an endotoxin lacking one or more secondary acyl chains of lipid A (*i.e.*, only penta- and tetraacylated lipid A).

There is simply no teaching in Karow *et al.* to suggest to those skilled in the art to make a mutation that results in an endotoxin that has a decreased number of acyl chains. One certainly would not have had a reasonable expectation that such an endotoxin would have substantially reduced toxicity. Not only must one have a motivation to try to make the invention, there must also be a reasonable expectation of success. Further, both the suggestion and the reasonable expectation of success must be found in the prior art, not in the applicant's disclosure.

Gupta *et al.* does not remedy the deficiencies of Karow *et al.* Gupta *et al.* disclose the conjugation of chemically-modified LPS to cholera toxin and other proteins. They do not, however, teach or suggest a method of making an endotoxin that has a decreased number of acyl chains.

Therefore, the present invention is not obvious over Karow *et al.* in view of Gupta *et al.*

G. Objections

The Examiner objected to claim 30 as grammatically incorrect in the recitation "comprising an active ingredient an htrB mutant". Claim 30 has been canceled, thereby rendering this objection moot.

CONCLUSION

Applicant believes the claims are in condition for allowance and request reconsideration of the application and allowance of the claims. The Examiner is invited to telephone the below-signed attorney at (612) 373-6961 to discuss any questions which may remain with respect to the present application.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 30 June 2000 By _____

Ann S. Viksnins
Reg. No. 37,748

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Box AF, Assistant Commissioner of Patents, Washington, D.C. 20231 on June 30, 2000.

Ann S. Viksnins _____

Name Signature

S/N 09/077,572

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al.
Serial No.: 09/077,572
Filed: October 13, 1998
Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Examiner: S. Devi
Group Art Unit: 1641
Docket: 875.001US2

DECLARATION OF DRS. GIBSON AND APICELLA UNDER 37 C.F.R. § 1.132

1. We, Bradford Gibson and Michael Apicella, are two of the co-inventors of the above-identified patent application.

2. I, Bradford Gibson, am currently a full professor of Pharmaceutical Chemistry and Chemistry at the University of California, San Francisco in the School of Pharmacy, where I have been since the Fall of 1985. I have worked in the area of biomedical mass spectrometry for 20 years after receiving my doctorate in chemistry from M.I.T. and a two year postdoctoral fellowship in the chemistry department, Cambridge University, UK. I have published numerous peer-reviewed publications on Lipid A and lipooligosaccharide (LOS) structures using mass spectrometry and NMR. I have collaborated with Dr. Michael Apicella for over 10 years on the structure analysis and biology of Lipid A's and LOS from numerous pathogenic and non-pathogenic bacteria including Haemophilus, Neisseria, Salmonella, E. coli and Moraxella.

3. I, Michael Apicella, am currently a tenured Professor and Chairman of Department of Microbiology at The University of Iowa in Iowa City, Iowa. I have held that position for seven years. I began my scientific career at Johns Hopkins University in 1966 and since that time have been a faculty member of the State University of New York at Buffalo and The University of Nevada, Reno. I obtained my M.D. degree from the State University of New York in Brooklyn in 1963. I have worked in the area of Bacterial pathogenesis and genetics for the past 30 years after completing my post-doctoral fellowship at Johns Hopkins School of Medicine. I have published over 140 articles in peer reviewed scientific journals in these areas since that time. As mentioned above, I have collaborated with Dr. Bradford Gibson for over 10 years on the structure analysis and biology of Lipid A's and LOS from numerous pathogenic and non-pathogenic bacteria including Haemophilus, Neisseria, Salmonella, E. coli and Moraxella.

4. We have performed studies that determined that *H. influenzae* makes a simple truncated penta- and tetraacylated lipid A, whose structure can be derived directly from the deletion of one or two O-linked myristoyl fatty acids (C_{14}) from the parental lipid A structure.

5. We have performed studies that determined that in wild type strains of *Neisseria gonorrhoeae*, lipid A is hexaacylated and contains two C-12 fatty acids (lauric acid), one on each of the two glucosamines. The *htrB* mutation in *N. gonorrhoeae* strain 1291 results in the complete deletion of one of these two lauric acid moieties to form a pentaacyl lipid A structure. No fully hexaacylated lipid A species is seen, nor higher mass structures or new fatty acids. The outcome for *htrB* in *N. gonorrhoeae* is similar to the *htrB* knockout in *H. influenzae*, which produced a truncated pentaacyl and tetraacyl lipid A species.

6. In addition, some changes in the phosphorylation pattern in the LOS and lipid A moiety are observed between wild type and *htrB*- mutant in *N. gonorrhoeae* strain 1291. These changes involve an increased level of phosphoethanolamine (PEA) in both the lipid A moiety as well as the oligosaccharide.

7. We obtained a culture of the *E. coli htrB* mutant (hereinafter "the Karow strain" or "the Karow mutant") from Costa Georgopoulos, one of the co-authors of the article Karow *et al.*, J. Bact., 174:7407-7418 (1992). We then performed studies on the lipid A made by the mutant strain. In particular, we performed a mass spectrometric examination of the Karow strain. The results of this examination clearly show that the Karow strain has a set of lipid A structures different in two very important ways from the *htrb* mutant pathogens of the present invention.

8. The Karow mutant makes a fully hexaacylated lipid A structure that is distinct in mass from the lipid A made by the parental wild-type strain. Specifically, the Karow mutant appears to contain a mixture of new unsaturated fatty acids, most likely palmitoleic (C16:1) in place of the single lauric acid (C12:0) fatty acid. This substitution causes a shift up in mass of 26 and 54 Da from the major wild type lipid A (molecular weight = 1798), producing new hexaacylated lipid A molecules with molecular weights of 1824 (+26, or C_2H_2) and 1852 (+ 54, or C_4H_6).

9. Even though pentaacyl and tetraacyl substituted lipid A species are seen in addition to hexaacyl structures in Karow's *E. coli*, these structures, when present, are not simple deletions of one and two fatty acids from the wild type (as is the case for *H. influenzae htrB*), but rather contain at least one new fatty acid not present in the small amounts of corresponding pentaacyl lipid A (MW = 1588, wild type pentaacyl lipid A) seen in the wild type lipid A preparation. The molecular weights of these two lipid A molecules are 1616 and 1406, and are consistent with a loss of the palmitoleic group (-236 Da, MW 1852--> 1616, mutant pentaacyl lipid A) and then a myristic acid group (-210 Da, MW 1616--> 1406, mutant tetraacyl lipid A).

10. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

May 8, 2000
Date

Bradford Gibson

June 29, 2000
Date

Michael Apicella

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

09/077,572

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
09/077,572	10/13/98	APICELLA M	875001US2

SCHWEGMAN LUNDBERG WOESSNER & KLUTH
PO BOX 2938
MINNEAPOLIS MN 55402

HM12/0824/JG100

JUN 11 2002
PATENT & TRADEMARK

EXAMINER	
DEVI, S	
ART UNIT	PAPER NUMBER
1645	23

DATE MAILED: 08/04/00

Below is a communication from the EXAMINER in charge of this application

COMMISSIONER OF PATENTS AND TRADEMARKS

D Brief Due - 2 Mo. - 8/30/00
Brief Due Deadline - 1/30/01

ADVISORY ACTION

☐ THE PERIOD FOR RESPONSE:

a) ☐ is extended to run _____ or continues to run _____ from the date of the final rejection

b) ☐ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for the response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition , and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date of the originally set shortened statutory period for response or as set forth in b) above.

☒ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☐ Applicant's response to the final rejection, filed 07.12.00 has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☒ The proposed amendments to the claim and /or specification will not be entered and the final rejection stands because:

   a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.

   b. ☒ They raise new issues that would require further consideration and/or search. (See Note).

   c. ☒ They raise the issue of new matter. (See Note).

   d. ☒ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.

   e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE: The new limitation of Int 13 mutant "pattern" introduced to claims 22 and 23 introduce new matter requiring a new rejection. Newly added claims 44 and 45 contain limitations that raise new issues and require further consideration and search.

2. ☐ Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ ~~Upon the filing of~~ A Notice of Appeal has been filed, the proposed amendment ☐ ~~will be entered~~ ☒ will not be entered and the status of the claims will be as follows:

Claims allowed: None.
Claims objected to: None.
Claims rejected: 22-26 and 29-31.

However;

☐ Applicant's response has overcome the following rejection(s): _____

4. ☐ The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection because _____

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficent reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☒ Other Interview Summary (paper no. 22).

TECH CENTER 1600/2900
JUN 14 2002

Schwegman, Lundberg, Woessner & Kluth, P.A.
AUG 14 2000
RECEIVED

LYNETTE R. F. SMITH
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Interview Summary

Application No.	Applicant(s)
09/077,572	Apicella et al.
Examiner	**Group Art Unit**
S. Devi, Ph.D.	1645

All participants (applicant, applicant's representative, PTO personnel):

(1) *S. Devi (PTO)* (3) _____

(2) *Ms. Ann Viksnins* (4) _____

Date of Interview *Aug 3, 2000*

Type: ☒ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☒ Yes ☐ No. If yes, brief description:
Applicants' After-Final Amendment.

Agreement ☐ was reached. ☐ was not reached.

Claim(s) discussed: *All of record.*

Identification of prior art discussed:

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:
A telephone message was left to Ms. Viksnins informing her of the reciept and non-entry of Applicants' After-Final amendment filed 07/12/00 since the newly added limitation to claims 22 and 23 add new matter and the newly added claims 44 and 45 contain limitations that require further consideration and search.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendents which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

SD 03 Aug 00

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

CONTINUED PROSECUTION APPLICATION (CPA)
UNDER 37 C.F.R. § 1.53(d)
REQUEST TRANSMITTAL

Address to:	*Attorney Docket No.:*	875.001US2
Commissioner for Patents **Box CPA** **Washington, D.C. 20231**	*First Named Inventor:*	Michael A. Apicella
	Express Mail No.:	EL709305825US
	Total Pages (if by fax):	

This is a request for filing a continuation application under 37 CFR § 1.53(d) of prior application Serial No. 09/077,572, filed on October 13, 1998, entitled NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA.

The above-identified prior application in which no abandonment of, or termination of, proceedings has occurred, is hereby expressly abandoned as of the filing date of this request for a CPA. Please use all the contents of the prior application file wrapper, including the drawings, as the basic papers for the new application. (37 CFR 1.53(b) must be used for continuation-in-part applications or for applications where the prior application is not to be abandoned.)

1. X Enter the amendment previously filed on June 30, 2000 under 37 CFR 1.116, but unentered, in the prior application.

2. __ A Preliminary Amendment (pages) is enclosed.

3. __ This application is filed by fewer than all the inventors named in the prior application, 37 CFR 1.53(d)(4).

   a. __ ***DELETE*** the following inventor(s) named in the prior nonprovisional application:

   _____

   b. __ The inventor(s) to be deleted are set forth on a separate sheet attached hereto.

4. __ A new power of attorney is enclosed.

5. __ Information Disclosure Statement is enclosed.
   a. __ Form(s) 1449
   b. __ Copies of IDS Citations

The filing fee is calculated below on the basis of the claims existing in the prior application as amended at 1 and 2 on the previous page:

	No. Filed	No. Extra	Rate	Fee
TOTAL CLAIMS	8 - 20 =	0	x 18 =	**$0.00**
INDEPENDENT CLAIMS	2 - 3 =	0	x 78 =	**$0.00**
[ ] MULTIPLE DEPENDENT CLAIMS PRESENTED				**$0.00**
BASIC FEE				**$690.00**
TOTAL				**$690.00**

6. __ Small Entity Status:

   a. __ A small entity statement is enclosed.

   b. __ A small entity statement was filed in the prior nonprovisional application and such status is still proper and desired.

   c. __ Is no longer claimed.

7. X A check in the amount of $690.00 is attached to pay the filing fee.

8. X **The Commissioner is hereby authorized to credit overpayments or charge any fees set forth in 37 CFR 1.16 through 1.18 to Deposit Account No. 19-0743.**

9. __ A petition for extension of time in the prior application is enclosed along with a check in the amount of $0.00 to pay the extension fee.

10. __ Other : _____

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)

By: _____
Atty: Ann S. Viksnins
Reg. No. 37,748

Customer Number 21186

"Express Mail" mailing label number: EL709305825US
Date of Deposit: August 16, 2000
This paper or fee is being deposited on the date indicated above with the United States Postal Service pursuant to 37 CFR 1.10, and is addressed to Box CPA, Commissioner for Patents, Washington, D. C. 20231.

875.001US2

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/077,572	10/13/98	AFICELLA	M 875001US2

HM12/1011
SCHWEGMAN LUNDBERG WOESSNER & KLUTH
PO BOX 2938
MINNEAPOLIS MN 55402

EXAMINER	
DEVI,S	
ART UNIT	PAPER NUMBER
1645	25

DATE MAILED: 10/11/00

OIPE JC100 JUN 11 2002 PATENT & TRADEMARK OFFICE

DV 11 JAN. 2001 – 3 MO.
11 APR. 2001 – 6 MO.

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

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JUN 14 2002
TECH CENTER 1600/2900

Schwegman, Lundberg,
Woessner & Kluth, P.A.

OCT 16 2000
RECEIVED

PTO-90C (Rev. 2/95)

2 - Mail Copy

Office Action Summary	Application No. 09/077,572	Applicant(s) Apicella et al.
	Examiner S. Devi, Ph.D.	Group Art Unit 1645

☒ Responsive to communication(s) filed on *Aug 16, 2000*.

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *22-26, 29, 32, and 33* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☒ Claim(s) *30 and 31* is/are ~~[illegible]~~ *cancelled*.

☒ Claim(s) *22-26, 29, 32, and 33* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

DETAILED ACTION

Change of Art Unit Location

1) Effective 20 June 2000, the Art Unit location of the instant application in the US PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Technology Center 1600, Group 1640, Art Unit 1645.

Request for Continued Prosecution Application

2) The request filed 08/16/2000 (paper no. 24) for a Continued Prosecution Application (CPA) under 37 C.F.R 1.53(d) based on the parent Application, SN 09/077,572, is acceptable and a CPA has been established. An action on the CPA follows.

Applicants' Amendment

3) Acknowledgment is made of Applicants' amendment filed 07/12/00 (paper no. 19) in response to the Final Office Action mailed 01/04/00 (paper no. 16).

Status of Claims

4) Claims 30 and 31 have been canceled via the amendment filed 06/12/00.

Claims 22, 23 and 29 have been amended via the amendment filed 06/12/00.

New claims 44 and 45 have been added via the amendment filed 06/12/00. These claims have been misnumbered. The numbering of claims is not in accordance with 37 CFR 1.126 which requires the original numbering of the claims to be preserved throughout the prosecution. When claims are canceled, the remaining claims must not be renumbered. When new claims are presented, they must be numbered consecutively beginning with the number next following the highest numbered claims previously presented (whether entered or not).

Misnumbered claims 44 and 45 have been renumbered as claims 32 and 33 respectively.

Claims 22-26, 29, 32 and 33 are pending and are under examination.

Declaration under 37 C.F.R § 1.132

5) Acknowledgment is made of Applicants' (Drs. Gibson and Apicella) declaration filed 07/12/00 (paper no. 9) under 37 C.F.R § 1.132.

Prior Citation of Title 35 Sections

6) The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office Action.

Prior Citation of References

7) The references cited or used as prior art in support of one or more rejections in the instant Office Action and not included on an attached form PTO-892 or form PTO-1449 have been previously cited and made of record.

Objection(s) Maintained

8) The objection to the drawings made in paragraph 6 of the Office Action mailed 04/28/99 (paper no. 11) under 37 CFR 1.84 because of the reasons set forth by the Draftsperson is maintained for reasons set forth therein. Applicants assure the Office that corrected formal drawings will be submitted upon notification of allowance of the claims.

Rejection(s) Moot

9) The rejection of claims 30 and 31 made in paragraph 16 of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, second paragraph, as being indefinite, is moot in light of Applicants' cancellation of the claims.

10) The rejection of claims 30 and 31 made in paragraph 17(e, f and g) of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, first paragraph, as being non-enabled with regard to the new matter issue, is moot in light of Applicants' cancellation of the claims.

11) The rejection of claim 31 made in paragraph 20 of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C § 103(a) as being anticipated by Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992), is moot in light of Applicants' cancellation of the claim.

Rejection(s) Withdrawn

12) The rejection of claim 29 made in paragraph 16 of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, first paragraph, as being non-enabled with regard to the new matter issue, is withdrawn in light of Applicants' amendment to the claim.

13) The rejection of claim 22 made in paragraph 17(a) of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

14) The rejection of claim 23 made in paragraph 17(c) of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

15) The rejection of claim 29 made in paragraph 17(d) of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

16) The rejection of claim 22 made in paragraph 17(e) of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

17) The rejection of claim 22 made in paragraph 19 of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C § 102(b) as being anticipated by Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) is withdrawn in light of Applicants' amendment to the claim.

18) The rejection of claims 23-26 made in paragraph 20 of the Office Action mailed 01/04/00 (paper no. 16) under 35 U.S.C § 103(a) as being anticipated by Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992) is withdrawn in light of Applicants' amendment to the claims or the base claim.

Rejection(s) Maintained

19) The rejection of claims 22, 23, 25 and 29 made in paragraph 9 of the Office Action mailed 04/28/99 (paper no. 11) under the judicially created provisional obviousness type double patenting is maintained for reasons set forth therein. Applicants state that if appropriate, they will consider filing a terminal disclaimer upon notification of allowable subject matter.

20) The rejection of claims 22-26 and 29 made in paragraph 10 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 112, first paragraph, with regard to the deposit of the mutant bacterium is maintained for reasons set forth therein. Applicants assure the Office that

upon receiving indication of allowable subject matter, Applicants will deposit plasmids pB28 and pB29 in compliance with 37 CFR 1.801-1.809.

The Applicants' Declaration under 37 C.F.R § 1.132

21) Applicants state that the Karow *htrB* mutant has a set of lipid A structures different in from the *htrB* mutant pathogens of the present invention as analyzed by a mass spectrometric examination. Applicants assert that the *htrB* mutation in *N. gonorrhoeae* strain 1291 results in the complete deletion of one of the two lauric acid moieties to form a pentaacyl lipid A structure and that the *htrB* knockout in *H. influenzae* produces a truncated pentaacyl and tetraacyl lipid A species. Applicants, however, acknowledge that Karow's *E. coli* mutant contains pentaacyl and tetraacyl substituted lipid A species, but contend that these structures contain at least one new fatty acid.

The information in the Applicants' Declaration has been carefully considered. However, as drafted currently, instant claims are obvious over the teachings of Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) in view of Westphal *et al.* and/or Gupta *et al.* as described below under art rejections. Instant claims, as drafted currently, do not contain, as limitations, the mass spectrometric differences found in the mutants of the instant invention compared to that of the prior art mutant or mutant endotoxin, i.e., Karow's mutant or mutant endotoxin. Instant claims do not include, as limitations, the presence only of truncated pentaacyl and tetraacyl lipid A species, the absence of fully acylated lipid A, and/or the absence of at least one new fatty acid in the instantly claimed mutants. Therefore, Karow *et al.* is a valid art and is properly applied to reject instant claims.

New Rejections

Applicants are asked to note the new rejections made in this Office Action. The Applicants' amendment including the addition of new claims, necessitated the new ground(s) of rejection presented in this Office Action.

Double Patenting

22) The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or

improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970) and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

23) Claim 32 is provisionally are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 25 and 27 of the copending Application, SN 09/565,943. Although the conflicting claims are not identical, they are not patentably distinct from each other. The invention claimed in the instant claim is encompassed in the scope of the above-mentioned claims of the co-pending application.

This is a provisional obviousness-type double patenting rejection, because the conflicting claims have not in fact been patented.

Rejection(s) under 35 U.S.C. § 112, First Paragraph

24) Claims 22, 29, 32 and 33 are rejected under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure, because the specification does not provide evidence that the biological materials of the claimed invention are (1) known and readily available to the public; (2) reproducible from the written description, e.g. sequenced; or (3) deposited.

It appears that the claimed *htrB* mutant Gram-negative bacterial pathogens as recited in instant claims are required to practice the claimed method of making and using the product, mutant endotoxin, of the instant invention. As required elements, the mutant bacteria must be known and readily available to the public, or obtainable by a reproducible method set forth in the specification. It is unclear if the mutant bacteria are publicly available, or can be reproducibly

isolated from nature without undue experimentation. Therefore, suitable deposits for patent purposes are suggested. The specification appears to lack complete deposit information for any of the *htrB* Gram-negative mutant bacterial pathogens that are specifically recited in instant claims. Without a publicly available deposit of the recited bacterial mutants, one of ordinary skill in the art could not be assured of the ability to practice the invention as claimed.

If the deposit is made under the terms of the Budapest Treaty, then an affidavit or declaration by Applicants, or a statement by an attorney of record stating that the deposit has been made under the terms of the Budapest Treaty and that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent on this application and that the deposit will be replaced if viable sample cannot be dispensed by the depository, is required to satisfy the deposit requirements. See 37 CFR 1.801-37 CFR 1.809. Further, the statement should identify the deposited mutant bacterial pathogens by their depository accession number, establish that the deposited mutant bacterial pathogens are the same as that described in the specification, and establish that the deposited bacterial pathogens were in Applicants' possession at the time of filing. *In re Lundak*, 773 F2d 1216, 227 USPQ 90 (Fed. Cir. 1985).

Rejection under 35 U.S.C. § 103(a)

25) Claims 22, 23, 25 and 32 are rejected under 35 U.S.C § 103(a) as being unpatentable over Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992, already of record) in view of Westphal *et al.* (*Methods Carbohydr. Chem.* 5: 83-91, 1965, already of record).

Karow *et al.* teach a method of making an endotoxin or LPS from a Gram negative bacterial pathogen, *E. coli*, containing a mutated *htrB* gene. The mutant bacterium produces a mutant endotoxin lacking one or more lauric acid and myristic acid (i.e., secondary acyl chains of lipid A) (see abstract; page 7413 left column; paragraph bridging left and right columns on page 7416, and page 7409, left column, under 'Fatty acid analysis'). The description provided in the Figure 4 legend indicates that the *htrB* mutant endotoxin is isolated from the *htrB* mutant bacterium (see page 7413). The lauric acid and myristic acid contents of the LPS form *htrB*

bacterial pathogen was reduced compared to that of wild type bacterial pathogen (see Figure 4 and page 7413). A method of making a *htrB* mutant of *E. coli* is also taught (see abstract and 'Materials and Methods'). That the absence of one or more lauric acid and myristic acid in the lipid A renders the bacterial LPS substantially less toxic compared to the wild type *E. coli* is inherent from the teachings of Karow *et al.*

Karow *et al.* do not expressly disclose a method of purifying the mutant endotoxin by phenol-water extraction, or a method of making of a *htrB* mutant of *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella or Pseudomonas,* or a *htrB* mutant endotoxin from these pathogens.

However, the method of purifying an endotoxin, for example, by phenol-water extraction is conventional and is well known in the art for decades. For instance, Westphal *et al.* teach phenol-water extraction of Gram negative bacterial lipopolysaccharides (see pages 86-90).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to purify Karow's *E. coli htrB* mutant endotoxin lacking one or more myristic acid substitutions in the lipid A using Westphal's phenol-water extraction method to produce the endotoxin of the instant invention, since Westphal's phenol-water extraction method is the widely used conventional method of purifying endotoxin. One skilled in the art would have had a reasonable expectation of success in obtaining a *htrB* mutant bacterium or a *htrB* mutant endotoxin from other Gram negative bacterial pathogens, such as, *Haemophilus*, *Neisseria*, *Moraxella*, *Campylobacter*, *Shigella* or *Pseudomonas* by extending Karow's method of making an *htrB* mutant *E. coli* bacterium or an *htrB E. coli* mutant endotoxin to one of these pathogens, since the lipid A parts of the LPS of *E. coli* and the Gram negative bacteria recited in claims 32 and 33 are structurally and/or biologically conserved with a similar genetic or biosynthetic make-up. Extending the Karow's method used for one Gram negative bacterial pathogen or its endotoxin, to another Gram negative pathogen or its endotoxin having a conserved lipid A would have been obvious to a skilled artisan and would have expected to bring about similar effects, absent evidence to the contrary.

Claims 22, 23, 25 and 32, as a whole, are obvious over the prior art of record.

26) Claims 24, 26, 29 and 33 are rejected under 35 U.S.C § 103(a) as being unpatentable over Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992, already of record) in view of Westphal *et al.* (*Methods Carbohydr. Chem.* 5: 83-91, 1965, already of record) as applied to claims 22, 23 and 25 above, and further in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992, already of record).

The teaching of Karow *et al.* as modified by Westphal *et al.* is explained above, which does not disclose conjugating the mutant endotoxin to a carrier protein, or raising antisera to the mutant endotoxin in an individual.

However, methods of conjugating a substantially less toxic endotoxin of a Gram negative bacterial pathogen to a carrier protein to enhance the immunogenicity of the endotoxin are well known and widely practiced in the art. For instance, Gupta *et al.* teach a method of conjugating a deacylated endotoxin of a Gram negative bacterial pathogen to a carrier protein to produce an immunogenic conjugate vaccine that can be used to raise endotoxin-specific antisera by administering it to an individual animal (see abstract, and pages 3202 and 3203).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to conjugate Karow's endotoxin as modified or purified by Westphal *et al.* to a carrier protein and raise endotoxin-specific antisera as taught by Gupta *et al.* One skilled in the art would have had a reasonable expectation of success in conjugating Karow's endotoxin as modified or purified by Westphal *et al.* to a carrier protein to produce a conjugate for use as a vaccine formulation, or as an immunogen to raise endotoxin-specific antisera of the instant invention, since the *htrB* mutant endotoxin lacking secondary acyl chains is expected to function significantly no differently in a conjugate than the deacylated endotoxin taught by Gupta *et al.* Extending such a method used for one Gram negative bacterial endotoxin, to another Gram negative pathogen endotoxin having a conserved lipid A, such as, those recited in claim 33 would have been obvious to a skilled artisan and would have expected to bring about similar effects, absent evidence to the contrary.

Claims 24, 26, 29 and 33, as whole, are *prima facie* obvious over the prior art of record.

Objection(s)

27) Claims 32 and 33 are objected to for the following reasons:

(a) Claims 32 and 33 are objected to for not italicizing the names of the bacterial genera: "Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella or Pseudomonas". To be consistent with the practice in the art, it is suggested that Applicants replace the recitation with -- *Haemophilus*, *Neisseria*, *Moraxella*, *Campylobacter*, *Shigella* or *Pseudomonas*--.

Remarks

28) Claims 22-26, 29, 32 and 33 stand rejected.

29) Papers related to this application may be submitted to Group 1600, AU 1645 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1 (CM1). The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242.

30) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. The Examiner can normally be reached on Monday to Friday from 8.00 a.m to 4.00 p.m.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

SD
S. Devi
Patent Examiner
September 2000

****
*** TX REPORT ***
****

TRANSMISSION OK

TX/RX NO	0330
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CONNECTION ID	TC 1600
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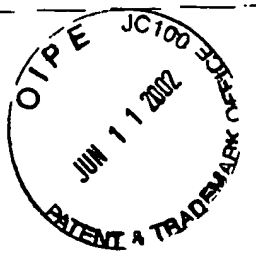

SCHWEGMAN ■ LUNDBERG ■ WOESSNER ■ KLUTH
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Minneapolis, MN 55402
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December 8, 2000

TO: Commissioner for Patents
Attn: S. Devi
Patent Examining Corps
Facsimile Center
Washington, D.C. 20231

FAX NUMBER (703) 308-4242

FROM: Ann S. Viksnins

OUR REF: 875.001US2

TELEPHONE: (612) 373-6961

* Please deliver to Examiner S. Devi in Art Unit 1645. *

THIS IS A FORMAL RESPONSE. Document(s) Transmitted:
Amendment and Response to Office Action mailed October 11, 2000 (6 pages)
Declaration Concerning Deposit of Microorganism (1 page)
ATCC Deposit Certificate (1 page)

Total pages of this transmission, including cover letter: 9 pgs
If you do NOT receive all of the pages described above, please telephone us at 612-373-6900, or fax us at 612-339-3061.

In re. Patent Application of: Michael A. Apicella et al.
Serial No.: 09/077,572
Filed: October 13, 1998
Examiner: S. Devi
Group Art Unit: 1645
Docket No.: 875.001US2

...ANTIGENIC DETERMINANTS OF PATHOGENIC GRAM NEGATIVE BACTERIA

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P.O. Box 2938
Minneapolis, MN 55402
Telephone (612) 373-6900 Facsimile (612) 339-3061

December 8, 2000

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THIS IS A FORMAL RESPONSE. Document(s) Transmitted:
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Total pages of this transmission, including cover letter: 9 pgs
If you do NOT receive all of the pages described above, please telephone us at 612-373-6900, or fax us at 612-339-3061.

In re. Patent Application of: Michael A. Apicella et al.
Serial No.: 09/077,572
Filed: October 13, 1998
Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Examiner: S. Devi
Group Art Unit: 1645
Docket No.: 875.001US2

Please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

By: _____
Name: Ann S. Viksnins
Reg. No.: Reg. No. 37,748

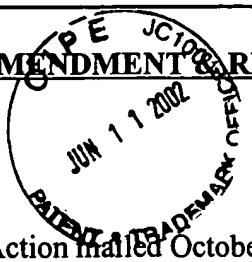
I hereby certify that this paper is being transmitted by facsimile to the U.S. Patent and Trademark Office on the date shown below.

Candy Buending

12/8/00
Date of Transmission

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1645
Filed:	October 13, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	



AMENDMENT & RESPONSE

Commissioner for Patents
Washington, D.C. 20231

In response to the Office Action mailed October 11, 2000, Applicant respectfully requests that the Examiner consider and enter the following amendments and remarks in connection with the above-identified patent application.

IN THE SPECIFICATION

At page 9, line 6, after "location." please insert --Nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29 were deposited on November 14, 2000 with the American Type Culture Collection,10801 University Blvd., Manassas, VA 20110-2209 under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application. Strain B28 has been accorded accession number PTA-2667 and strain B29 has been accorded accession number PTA-2668--.

IN THE CLAIMS

Please amend the claims as follows. For the Examiner's convenience, all pending claims are presented below.

22. [Amended] A method of making a mutant endotoxin comprising

mutating an *htrB* gene within a gram-negative bacterial pathogen to form an *htrB* mutant pathogen, wherein the *htrB* gene encodes an endotoxin lacking one or more secondary acyl chains of lipid A contained in a wild type gram-negative bacterial pathogen and lacking 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen, and wherein the mutant endotoxin has

substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen, and

purifying the mutant endotoxin from the *htrB* mutant pathogen.

23. A mutant endotoxin made according to the method of claim 22, wherein the mutant endotoxin was purified from the *htrB* mutant pathogen by phenol-water extraction or by protease digestion.

24. The mutant endotoxin according to claim 23, wherein the mutant endotoxin is conjugated to a carrier protein.

25. A mutant endotoxin made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, wherein the mutant endotoxin is conjugated to a carrier protein.

29. [Amended] A method for producing endotoxin-specific antisera, the method comprising

(a) immunizing an individual with a vaccine formulation comprising an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, or endotoxin purified from the *htrB* mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the *htrB* mutant lacks one or more secondary acyl chains of lipid A contained in a wild type gram-negative bacterial pathogen and lacks 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.

32. [Amended] The method of claim 22 wherein the gram-negative bacterial pathogen is of the genera [Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella or Pseudomonas] *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella* or *Pseudomonas*.

33. [Amended] The method of claim 29 wherein the gram-negative bacterial pathogen is of the genera [Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella or Pseudomonas] *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella* or *Pseudomonas*.

REMARKS

A. Status of Claims

Reconsideration of this application as amended is requested. Claims 22, 29 and 32-33 having been amended, claims 22-26, 29 and 32-33 are pending. No new subject matter has been added.

The amendments to the claims are fully supported by the specification as originally filed. The amendments are made to clarify the claims, and are not intended to limit the scope of equivalents to which any claim element may be entitled. For example, support for the amendments to claims 22 and 29 regarding the length of the secondary acyl chain is found at page 7, lines 23-26, and at page 13, lines 1-5 of the specification, and in Figures 2A and 2B.

B. Rejection of Claims under 35 U.S.C. §112, First Paragraph

Applicant acknowledges that the Examiner has maintained the rejection of claims 22-26 and 29 under 35 U.S.C. § 112, first paragraph and that the rejection will be withdrawn upon the receipt of the required deposit information.

Enclosed herewith is a copy of the deposit receipts and viability statements from the ATCC regarding Nontypeable *Haemophilus influenzae* 2019 B28 and Nontypeable *Haemophilus influenzae* 2019 B29. Also enclosed is a Declaration by Dr. Apicella indicating that the strains described in the specification were deposited under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application, and the deposits will be replaced if viable samples cannot be dispensed by the depository. The Declaration also states that the strains described in the specification as filed are the same as the

strains deposited in the depository, and the deposited strains were in Applicants' possession at the time of filing of the above-identified application. Therefore, this rejection under 35 U.S.C. § 112, first paragraph should be withdrawn.

C. Non-Statutory Double Patenting Rejection

The Examiner provisionally rejected the pending claims under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 22, 23, 25 and 29 of U.S. Patent Application No. 08/565,943. Applicants will consider filing a terminal disclaimer upon notification of otherwise allowable subject matter. A terminal disclaimer may not be appropriate once the scope of allowable claims is determined in the present application, and dependent upon which application is allowed first.

D. Objections to the Claims

Claims 32 and 33 were objected to for minor stylistic reasons. These claims have been amended to overcome these rejections.

F. Rejections under 35 U.S.C. §103(a)

The Examiner has rejected claims 22, 23, 25 and 32 under 35 U.S.C. §103(a) as being unpatentable over Karow *et al.*, (*Journal of Bacteriology* 174:7407-7418) in view of Westphal *et al.* (*Methods Carbonydr. Chem.* 5:83-91, 1965). This rejection is respectfully traversed.

The claims as amended recite a method of making a mutant endotoxin that lacks at least one secondary acyl chain on lipid A and that lacks a 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen. This is clearly distinguishable over Karow *et al.*

First, the Karow *et al.* mutant makes a fully hexaacylated lipid A structure. §132 Declaration, ¶ 8 (filed on June 30, 2000). The organism of the present invention contains only lipid A structures that lack at least one secondary acyl chain on lipid A. Second, the Karow *et al.* mutant contains a mixture of new unsaturated fatty acids, most likely palmitoleic (C16:1) in place of the single lauric acid (C12:0) fatty acid. *Id.* The lipid A species of the present invention lacks a 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-

type bacterial pathogen. Therefore, significant differences exist in the lipid A structures in the *htrB* gene deletion mutants of the present invention as compared to Karow's strain.

Westphal *et al.* does not remedy the deficiencies of Karow *et al.* Westphal *et al.* disclose a method of purifying Gram negative bacterial lipopolysaccharides by phenol-water extraction. They do not, however, teach or suggest a method of making an endotoxin of the present invention, *i.e.*, one that lacks at least one secondary acyl chain on lipid A and that lacks a 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen.

Therefore, the present invention is not obvious over Karow *et al.* in view of Westphal *et al.* and this rejection under 35 U.S.C. §103(a) should be withdrawn.

The Examiner has rejected claims 24, 26, 29 and 33 under 35 U.S.C. §103(a) as being unpatentable over Karow *et al.*, (*Journal of Bacteriology* 174:7407-7418) in view of Westphal *et al.* (*Methods Carbonydr. Chem.* 5:83-91, 1965), and further in view of Gupta *et al.* (*Infect. Immun.* 60: 3201-3208, 1992). This rejection is respectfully traversed.

Karow *et al.* and Westphal *et al.* have been discussed above. Gupta *et al.* does not remedy the deficiencies of Karow *et al.* and Westphal *et al.* Gupta *et al.* disclose the conjugation of chemically-modified LPS to cholera toxin and other proteins. They do not, however, teach or suggest a method of making an endotoxin that lacks at least one secondary acyl chain on lipid A and that lack 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen.

Therefore, the present invention is not obvious over Karow *et al.* in view of Gupta *et al.* and this rejection under 35 U.S.C. §103(a) should be withdrawn.

CONCLUSION

Applicant believes that all claims are in condition for allowance. Reconsideration of the rejections of the claims and allowance of all the claims is respectfully requested. The Examiner is invited to contact the Applicant's attorney if prosecution of the present application can be assisted thereby.

Respectfully submitted,

MICHAEL A. APICELLA *ET AL.*

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 8 December 2000 By _____
Ann S. Viksnins
Reg. No. 37,748

Certificate Under 1.6: I hereby certify that this paper is being transmitted by facsimile to the U.S. Patent and Trademark Office, Attn.: Examiner Devi, Group 1645, on the date shown below.

_____ Date: December 8, 2000
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S/N 09/077,572 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1645
Filed:	October 13, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

DECLARATION CONCERNING DEPOSIT OF MICROORGANISMS

I, Michael A. Apicella, declare and say as follows:

1. I am an inventor with respect to the above-identified application.

2. On November 14, 2000, I made a patent deposit of nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29 to the American Type Culture Collection Collection,10801 University Blvd., Manassas, VA 20110-2209 under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application, and the deposits will be replaced if viable samples cannot be dispensed by the depository. Strain 2019 B28 was accorded ATCC Deposit number PTA-2667. Strain B29 was accorded ATCC Deposit number PTA-2668. The *Haemophilus* strains described in the specification as filed are the same as the *Haemophilus* strains deposited in the depository, and the deposited *Haemophilus* strains were in Applicants' possession at the time of filing of the above-identified application.

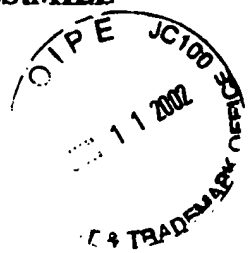
3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

12/8/00
Date

Michael A. Apicella
Michael A. Apicella

AMERICAN TYPE CULTURE COLLECTION
10801 University Blvd.
Manassas, VA 20110-2209
Telephone: 703-365-2700
Fax: 703-365-2745

FACSIMILE



Date: November 17, 2000

To: Ann S. Viksnins
Fax Number: 612 339-3061

From: ATCC Patent Depository **Number of pages:** 1 **(Including this page)**

REFERENCE: **Patent Deposit (Ref: Docket or Case No. 875.001US2-UIRF N5-50)**

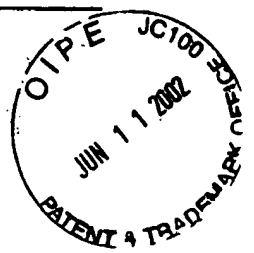
Nontypeable Haemophilus influenzae: 2019 B28 assigned PTA-2667 and
Nontypeable Haemophilus influenzae: 2019 B29 assigned PTA-2668

Date of Deposit: November 14, 2000. Paperwork will be forwarded to you in a few days. An invoice will be sent under separate cover referencing the VISA account of Suzanne Hoofnagle:

Standard storage/informing	$ 2,200.00
Viability Test	300.00
Total amount to PTA-2667 and PTA-2668	$ 2,500.00

Marie Harris

Marie Harris, Patent Specialist
ATCC Patent Depository



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VB

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/077,572	10/13/98	APICELLA	M 875001US2

HM12/0221
SCHWEGMAN LUNDBERG WOESSNER & KLUTH
PO BOX 2938
MINNEAPOLIS MN 55402

EXAMINER	
DEVI,S	
ART UNIT	**PAPER NUMBER**
1645	27

DATE MAILED: 02/21/01

April 21, 2001
MAY 21, 2001
Aug. 21, 2001

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/077,572	Apicella et al.
Examiner	**Group Art Unit**
S. Devi, Ph.D.	1645

☒ Responsive to communication(s) filed on *12/08/2000.*

☒ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *22-26, 29, 32, and 33* is/~~are~~ pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *22-26, 29, 32 and 33* is/~~are~~ rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

DETAILED ACTION

Applicants' Amendment

1) Acknowledgment is made of Applicants' amendment filed 12/08/00 (paper no. 26) in response to the non-final Office Action mailed 10/11/00 (paper no. 25). Amendment requested to be introduced to page 9, line 6 of the specification has NOT been entered, since there is no word "location" on page 9, line 6.

Status of Claims

2) Claims 22, 29, 32 and 33 have been amended via the amendment filed 12/08/00.

Claims 22-26, 29, 32 and 33 are pending and are under examination.

Objection(s) Maintained

3) The objection to the drawings made in paragraph 6 of the Office Action mailed 04/28/99 (paper no. 11) under 37 CFR 1.84 because of the reasons set forth by the Draftsperson is maintained for reasons set forth therein. Applicants assure the Office that corrected formal drawings will be submitted upon notification of allowance of the claims.

Objection(s) Withdrawn

4) The objection to claims 32 and 33 made in paragraph 27 of the Office Action mailed 10/11/00 (paper no. 25) is withdrawn in light of Applicants' amendments to the claims.

Rejection(s) Maintained

5) The rejection of claims 22, 23, 25 and 29 made in paragraph 9 of the Office Action mailed 04/28/99 (paper no. 11) under the judicially created provisional obviousness type double patenting is maintained for reasons set forth therein. Applicants state that if appropriate, they will consider filing a terminal disclaimer upon notification of allowable subject matter.

6) The rejection of claims 22-26 and 29 made in paragraph 10 of the Office Action mailed 04/28/99 (paper no. 11) under 35 U.S.C. § 112, first paragraph, with regard to the deposit of the mutant bacterium is maintained for reasons set forth therein. Applicants have submitted a copy of ATCC deposit receipt showing that non-typeable *Haemophilus influenzae* 2019 B28 and 2019 strains have been deposited under the provisions of the Budapest Treaty and provided the

statement that all restrictions will be irrevocably removed upon the granting of a patent in compliance with 37 CFR 1.801-1.809. However, the amendment requested by Applicants at page 9, line 6 after the recitation "location" remains unentered, since there is no such recitation on page 9, line 6. Since Applicants have not fully complied with 37 C.F.R 1.801-1.809, the rejection will be maintained currently.

7) The rejection of claim 32 made in paragraph 23 of the Office Action mailed 10/11/00 (paper no. 25) under the judicially created provisional obviousness type double patenting over the cited claim(s) of application SN 09/565,943 is maintained for reasons set forth therein. Applicants state that if appropriate, they will consider filing a terminal disclaimer upon notification of allowable subject matter.

8) The rejection of claims 22, 29, 32 and 33 made in paragraph 24 of the Office Action mailed 10/11/00 (paper no. 25) under 35 U.S.C. § 112, first paragraph, as failing to provide an enabling disclosure, with regard to the deposit issue, is maintained for reasons set forth above in paragraph 6.

Rejection(s) Withdrawn

9) The rejection of claims 22, 23, 25 and 32 made in paragraph 25 of the Office Action mailed 10/11/00 (paper no. 25) under 35 U.S.C. § 103(a) as being unpatentable over Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) in view of Westphal *et al.* (*Methods Carbohydr. Chem.* 5: 83-91, 1965), is withdrawn in light of Applicants' amendments to the base claim.

10) The rejection of claims 22, 23, 25 and 32 made in paragraph 26 of the Office Action mailed 10/11/00 (paper no. 25) under 35 U.S.C. § 103(a) as being unpatentable over Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992) in view of Westphal *et al.* (*Methods Carbohydr. Chem.* 5: 83-91, 1965) is withdrawn in light of Applicants' amendments to the base claim.

New Rejection(s)

Applicants are asked to note the new rejections made in this Office Action. The Applicants' amendment necessitated the new grounds of rejections presented in this Office Action.

Rejection(s) under 35 U.S.C. 112, First paragraph

11) Claims 22-26, 29, 32 and 33 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Base claims 22 and 29 currently include the limitation "and lacking 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen". However, there appears to be no descriptive support in the instant specification for the newly added limitation. Applicants have pointed to page 7, lines 23-26, page 13, lines 1-5 and Figures 2A and 2B of the specification as supporting the newly added limitation or amendment to the claims. However, these parts of the specification do not provide descriptive support for the newly added limitation.

The new limitation in the claims is therefore considered to be new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P 608.04 to 608.04(c).

Applicants are respectfully requested to point exactly to the descriptive support in the specification as filed, for the newly added limitation, or to remove the new matter from the claims.

Remarks

12) Claims 22-26, 29, 32 and 33 stand rejected.

13) **THIS ACTION IS MADE FINAL.** Applicants are reminded of the extension of time policy as set forth in 37 C.F.R 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

14) Papers related to this application may be submitted to Group 1600, AU 1645 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1 (CM1). The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242, which receives papers 24 hours a day, seven days a week.

15) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. A telephone message may be left on the Examiner's voice mail system. The Examiner can normally be reached on Monday to Friday from 7.15 a.m to 4.15 p.m. except one day each bi-week which would be disclosed on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

SD
S. Devi, Ph.D.
Patent Examiner
February 2001

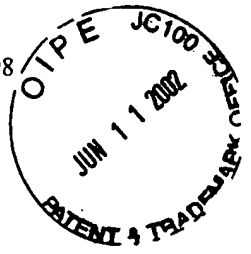
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al.

Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Docket No.: 875.001US2
Filed: October 13, 1998
Examiner: S. Devi

Serial No.: 09/077,572
Due Date: April 21, 2001
Group Art Unit: 1645



BOX AF
Commissioner for Patents
Washington, D.C. 20231

We are transmitting herewith the following attached items (as indicated with an "X"):

X A return postcard.
X An Amendment and Response Under 37 C.F.R. 1116 (10 Pages).
X Copy of *Ex parte Parks*

Please consider this a PETITION FOR EXTENSION OF TIME for sufficient number of months to enter these papers and please charge any additional required fees or credit overpayment to Deposit Account No. 19-0743.

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)

By: _____
Atty: Ann S. Viksnins
Reg. No. 37,748

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: BOX AF, Commissioner for Patents, Washington, D.C. 20231, on this 16th day of March, 2001.

Cindy Brandino
Name

_____
Signature

Customer Number 21186
SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A. P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)
(GENERAL)

EXPEDITED PROCEDURE - EXAMINING GROUP 1645

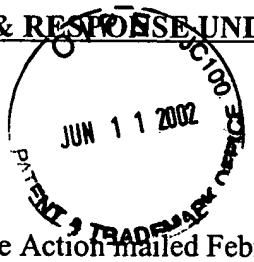
S/N 09/077,572 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi
Serial No.:	09/077,572	Group Art Unit: 1645
Filed:	October 13, 1998	Docket: 875.001US2
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA	

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116

Box AF
Commissioner for Patents
Washington, D.C. 20231



In response to the final Office Action mailed February 21, 2001, Applicant respectfully requests that the Examiner consider and enter the following amendments and remarks in connection with the above-identified patent application.

IN THE SPECIFICATION

Please delete the paragraph beginning on page 13 at line 27 and ending on page 14 at line 8, and insert the following paragraph therefor:

--Two plasmids, termed pB28 and pB29, each with a mini-Tn3 transposon containing the chloramphenicol acetyltransferase (CAT) gene inserted into the *htrB* open reading frame at a different location. Nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29 were deposited on November 14, 2000 with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209 under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application. Strain B28 has been accorded accession number PTA-2667 and strain B29 has been accorded accession number PTA-2668. Each plasmid was used to transform nontypeable *H. influenzae* strain 2019 and bacterial cell transformants were selected for by growth in the presence of chloramphenicol (1.5 μg/ml), resulting in identification of mutant strains designated NTHi B28 and B29, respectively. Locations of the mTn3 insertion in the chromosomes of the NTHi mutants were confirmed by genomic Southern hybridization using the 2.4 kb *Bgl*II fragment as a probe. In particular, a *Bgl*II digest of NTHi strain 2019 DNA resulted in a 2.4 kb fragment; whereas

similar digests of DNA from mutants NTHi B28 and B29 revealed 4.0 kb fragments. Further, the 4.0 kb fragments were digested by *Eco*RI which is present in the mTn3.--

A clean copy of this paragraph is attached hereto.

IN THE CLAIMS

Please substitute the claim set in the appendix entitled Clean Version of Pending Claims for the previously pending claim set. Specific amendments to individual claims are detailed in the following marked up set of claims.

Please add new claim 34 and amend the claims as follows.

22. (Amended) A method of making a mutant endotoxin comprising

mutating an htrB gene encoding a wild type endotoxin in [within] a wild type gram-negative bacterial pathogen to provide the mutant endotoxin; wherein the mutant endotoxin is the same as the wild type endotoxin except for [form an htrB mutant pathogen, wherein the htrB gene encodes an endotoxin] lacking one or more secondary acyl chains of lipid A [contained in a wild type gram-negative bacterial pathogen and lacking 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen], and wherein the mutant endotoxin has substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen[, and

purifying the mutant endotoxin from the htrB mutant pathogen].

29. (Amended) A method for producing endotoxin-specific antisera, the method comprising

(a) immunizing an individual with a vaccine formulation comprising an htrB mutant of a gram-negative bacterial pathogen, endotoxin isolated from the htrB mutant of the gram-negative bacterial pathogen, or endotoxin purified from the htrB mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the htrB mutant endotoxin is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A [lacks one or more secondary acyl chains of lipid A contained in a wild type gram-negative bacterial pathogen and lacks 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen].

34. (New) The method of claim 22, further comprising the step of purifying the mutant endotoxin.

REMARKS

A. Status of Claims

Reconsideration of this application as amended is requested. Claims 22 and 29 having been amended, claim 34 being newly added, claims 22-26, 29 and 32-34 are pending. No new subject matter has been added.

The amendments to the claims are fully supported by the specification as originally filed. The amendments are made to clarify the claims, and are not intended to limit the scope of equivalents to which any claim element may be entitled. Support for new claim 34 is found in original claim 22. Support for the amendments to claims 22 and 29 is found throughout the specification. One having ordinary skill in the art upon reading the full disclosure would recognize that the claimed mutant endotoxin is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A, i.e., only one change is made between the wild type and mutant endotoxin, and that change is the number of acyl chains in the lipid A. For example, Figure 1 depicts a wild type endotoxin (hexaacyl), and Figures 2A and 2B depict mutant endotoxin (pentaacyl and tetraacyl, respectively). See also Brief Description of the Figures on page 4 of the specification. The only change between Figure 1 and Figures 2A/2B is a decrease in the number of secondary acyl chains. There is no other change in the lipid A (such as length of the remaining chains). Further, page 4, lines 3-9 of the specification states that the lipid A produced by the mutant lacks one or both of the fatty acids, thereby rendering the endotoxin substantially reduced in toxicity, and yet retaining antigenicity as compared to wild

type. Page 7, lines 7-10 states that the mutants specifically lack one or more secondary acyl chain fatty acids that are ester-bound to the hydroxyl grous of two of the four molecules of β-OH. Moreover, on page 13, lines 1-5 of the specification states that the lipid A structure of the mutant endotoxin has one or two fewer acyl chains than the wild type.

It should be noted that "adequate description under the first paragraph of 35 U.S.C. §112 does not require *literal* support for the claimed invention." (emphasis in original) *Ex parte Parks*, 30 USPQ2d 1234-1237, 1236 (Bd. Pat App. 1993); *citing In re Herschler*, 591 F.2d 693, 200 USPQ 711 (CCPA 1979); *In re Edwards*, 568 F.2d 1349, 196 USPQ 465 (CCPA 1978); In re Werthein, 541 F.2d 257, 191 USPQ 90 (CCPA 1976). Rather, it is sufficient if the originally-filed disclosure would have conveyed to one having ordinary skill in the art that an appellant had possession of the concept of what is claimed. *In re Anderson*, 471 F.2d 1237, 176 USPQ 331 (CCPA 1973). As discussed above, clearly one with ordinary skill in the art upon reading the full specification would understand that the claimed mutant endotoxin is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A. Therefore, the claims as currently amended meet the adequate description requirement of 35 U.S.C. §112, first paragraph.

B. Rejections of Claims under 35 U.S.C. §112, First Paragraph

1. Deposit of Microorganisms

Applicant acknowledges that the Examiner has maintained the rejection of claims 22-26 and 29 under 35 U.S.C. § 112, first paragraph and that the rejection will be withdrawn upon the receipt of the required deposit information.

A copy of the deposit receipts and viability statements from the ATCC regarding Nontypeable *Haemophilus influenzae* 2019 B28 and Nontypeable *Haemophilus influenzae* 2019 B29 were submitted along with the Amendment dated December 8, 2000. Also enclosed with the December 8, 2000 Amendment was a Declaration by Dr. Apicella indicating that the strains described in the specification were deposited under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application, and the deposits will be replaced if viable samples cannot be dispensed by the depository. The Declaration also stated that the strains described in the specification as filed are the same as the

strains deposited in the depository, and the deposited strains were in Applicants' possession at the time of filing of the above-identified application. Therefore, this rejection under 35 U.S.C. § 112, first paragraph should be withdrawn.

2. Written Description

The Examiner has rejected claims 22-26, 29, 32 and 33 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention. In particular, the Examiner objected to the phrase "lacking 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen". Applicant has now amended the claims to delete this phrase. Therefore, this rejection is rendered moot, and should be withdrawn.

C. Non-Statutory Double Patenting Rejection

The Examiner provisionally rejected the pending claims under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 22, 23, 25 and 29 of U.S. Patent Application No. 08/565,943. Applicants will consider filing a terminal disclaimer upon notification of otherwise allowable subject matter. A terminal disclaimer may not be appropriate once the scope of allowable claims is determined in the present application, and dependent upon which application is allowed first.

D. Objection to the Drawings

Corrected formal drawings will be submitted upon notification of allowance of the claims.

E. Distinction of Pending Claims over Previously-Cited Art

1. Karow et al. and Westphal et al.

The pending claims are distinguishable over Karow et al., (*Journal of Bacteriology* 174:7407-7418) in view of Westphal et al. (*Methods Carbonydr. Chem.* 5:83-91, 1965).

The claims as amended recite a method of making a mutant endotoxin, wherein the mutant endotoxin *is the same as* the wild type endotoxin except for lacking one or more secondary acyl chains of lipid A This is clearly distinguishable over Karow et al.

The mutant E. coli identified by Karow et al. makes a set of lipid A structures different from the mutants of the present invention. First, the Karow culture made a fully hexaacylated lipid A structure. §132 Declaration, ¶ 8 (filed on June 30, 2000). The present invention does not include a hexaacylated lipid A structure from *E. coli*. Second, the Karow et al. *E. coli* made an endotoxin containing fewer than six acylated lipid A fatty acids but additionally had changes in the length of the other fatty acid chains. *Id.* For example, the Karow et al. mutant contained a mixture of new unsaturated fatty acids, most likely palmitoleic (C16:1) in place of the single lauric acid (C12:0) fatty acid. *Id.* The lipid A species of the present invention does not contain these changes; the mutant endotoxin of the present invention *is the same as* the wild type endotoxin except for lacking one or more secondary acyl chains of lipid A. Thus, significant differences exist in the lipid A structures in the *htrB* gene deletion mutants of the present invention as compared to Karow's strain. There is simply no teaching in Karow et al. to suggest to those skilled in the art to make a mutation that results in the lipid A recited in the present claims.

Westphal et al. does not remedy the deficiencies of Karow et al. Westphal et al. disclose a method of purifying Gram negative bacterial lipopolysaccharides by phenol-water extraction. They do not, however, teach or suggest a method of making an endotoxin of the present invention, i.e., method of making a mutant endotoxin, wherein the mutant endotoxin *is the same as* the wild type endotoxin except for lacking one or more secondary acyl chains of lipid A

Therefore, the present invention is not obvious over Karow et al. in view of Westphal et al.

2. Karow et al. in view of Westphal et al. and Gupta et al.

The pending claims are distinguishable over Karow et al., (*Journal of Bacteriology* 174:7407-7418) in view of Westphal et al. (*Methods Carbonydr. Chem.* 5:83-91, 1965), and further in view of Gupta et al. (Infect. Immun. 60: 3201-3208, 1992).

Karow et al. and Westphal et al. have been discussed above. Gupta et al. does not remedy the deficiencies of Karow et al. and Westphal et al. Gupta et al. disclose the conjugation of

chemically-modified LPS to cholera toxin and other proteins. They do not, however, teach or suggest a method of making a mutant endotoxin, wherein the mutant endotoxin is the same as the wild type endotoxin except for lacking one or more secondary acyl chains of lipid A.

Therefore, the present invention is not obvious over Karow et al. in view of Westphal et al. and Gupta et al.

CONCLUSION

Applicant believes that all claims are in condition for allowance. Reconsideration of the rejections of the claims and allowance of all the claims is respectfully requested. The Examiner is invited to contact the Applicant's attorney if prosecution of the present application can be assisted thereby.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER
& KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6961

Date 16 March 2001 By _____

Ann S. Viksnins
Reg. No. 37,748

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Box AF, Commissioner of Patents, Washington, D.C. 20231, on this 16th day of March, 2001.

Cindy Brendig
Name

_____
Signature

Clean copy of replacement for the paragraph in the specification beginning on page 13, line 27, and ending on page 14, line 8:

Two plasmids, termed pB28 and pB29, each with a mini-Tn3 transposon containing the chloramphenicol acetyltransferase (CAT) gene inserted into the *htrB* open reading frame at a different location. Nontypeable *Haemophilus influenza* strains 2019 B28 and 2019 B29 were deposited on November 14, 2000 with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209 under the provisions of the Budapest Treaty, and all restrictions will be irrevocably removed upon the granting of a patent on this application. Strain B28 has been accorded accession number PTA-2667 and strain B29 has been accorded accession number PTA-2668. Each plasmid was used to transform nontypeable *H. influenzae* strain 2019 and bacterial cell transformants were selected for by growth in the presence of chloramphenicol (1.5 μg/ml), resulting in identification of mutant strains designated NTHi B28 and B29, respectively. Locations of the mTn3 insertion in the chromosomes of the NTHi mutants were confirmed by genomic Southern hybridization using the 2.4 kb *Bgl*II fragment as a probe. In particular, a *Bgl*II digest of NTHi strain 2019 DNA resulted in a 2.4 kb fragment; whereas similar digests of DNA from mutants NTHi B28 and B29 revealed 4.0 kb fragments. Further, the 4.0 kb fragments were digested by *Eco*RI which is present in the mTn3.

Clean copy of the pending claims 22-26, 29, and 32-34

22. (Amended) A method of making a mutant endotoxin comprising

mutating an *htrB* gene encoding a wild type endotoxin in a wild type gram-negative bacterial pathogen to provide the mutant endotoxin; wherein the mutant endotoxin is the same as the wild type endotoxin except for lacking one or more secondary acyl chains of lipid A, and wherein the mutant endotoxin has substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen.

23. A mutant endotoxin made according to the method of claim 22, wherein the mutant endotoxin was purified from the *htrB* mutant pathogen by phenol-water extraction or by protease digestion.

24. The mutant endotoxin according to claim 23, wherein the mutant endotoxin is conjugated to a carrier protein.

25. A mutant endotoxin made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, wherein the mutant endotoxin is conjugated to a carrier protein.

29. (Amended) A method for producing endotoxin-specific antisera, the method comprising

(a) immunizing an individual with a vaccine formulation comprising an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, or endotoxin purified from the *htrB* mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the *htrB* mutant endotoxin is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A.

32. The method of claim 22 wherein the gram-negative bacterial pathogen is of the genera *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella* or *Pseudomonas*.

33. The method of claim 29 wherein the gram-negative bacterial pathogen is of the genera *Haemophilus, Neisseria, Moraxella, Campylobacter, Shigella* or *Pseudomonas*.

34. (New) The method of claim 22, further comprising the step of purifying the mutant endotoxin.

years' worth of license fees, or $1,260, since the date of its first letter to defendants on September 23, 1933 informing them that they were required to sign a license agreement. By imposing the statutory minimum of $500 per number of works infringed,[1] defendants will be required to pay $11,500, approximately nine times the amount defendants would have paid in licensing fees. This Court finds that to be an appropriate penalty for the defendants' infringements.

Finally, the Copyright Act provides that the court "in its discretion may allow the recovery of full costs [and] may also award a reasonable attorney's fee to the prevailing party as part of the costs." 17 U.S.C. § 505. In order to encourage suits to redress copyright infringement, attorney fees are awarded to a prevailing plaintiff as a matter of course. *Frost Belt Int'l Recording Enterprises, Inc. v. Cold Chillin' Records*, 758 F.Supp. 131, 140 (S.D.N.Y. 1990) The award of attorney's fees is the rule rather than the exception. *Micromanipulator Co. v. Bough*, 779 F.2d 255, 259 [228 USPQ 443] (5th Cir. 1985). Consequently, this Court finds plaintiffs entitled to reasonable attorney's fees for the prosecution of this action.

The declaration of Marjorie R. Esman submitted by plaintiffs states that plaintiffs incurred $1,747.00 in attorney's fees for services, including: preparation and service of discovery materials, participation in a scheduling conference; preparation of and filing of a witness and exhibit list; preparation and filing of the motion for summary judgment. The declaration states that plaintiffs incurred costs and expenses in the amount of $485.37 for filing of the complaint, payments to the process server, reasonable photocopies, and long distance telephone charges. This Court finds these declared attorneys's fees, costs and expenses to be reasonable.

Conclusion

For the reasons set forth above,

IT IS ORDERED that plaintiffs' motion for summary judgment is hereby GRANTED in all respects except plaintiffs' request for statutory damages in the amount of $2,500 per claim of infringement. Accordingly, defendants are liable to plaintiffs in the amount of $11,500 in statutory damages for copyright infringements, $1,747.00 in attorney's fees, and $485.37 in costs and expenses. Judgment will be so entered.

[1] See *Frank Music Corp. v. Metro-Goldwyn-Mayer Inc.*, (9th Cir.), 886 F.2d 1545 [12 USPQ2d 1412], *cert. den'd* 110 S.Ct. 1321, 494 U.S. 1017 (1989) which states that the number of works infringed is the appropriate calculation for statutory damages and not the number of infringements. The affidavit of James Hutcherson, investigator for BMI, lists 23 works which were infringed on July 11, 12, 18, and 19, 1992.

U.S. Patent and Trademark Office
Board of Patent Appeals and Interferences

Ex parte Parks

No. 93-2740

Decided September 2, 1993
Released January 4, 1994

PATENTS

1. Practice and procedure in Patent and Trademark Office — Reissue — Broader claims sought (§110.1313)

Patentability/Validity — Specification — Written description (§115.1103)

Claims in reissue application for method of determining nitrogen content of sample were improperly rejected on ground of inadequate descriptive support under 35 USC 112, first paragraph, since originally-filed disclosure need only convey, to one of skill in art, that applicant had possession of concept of what is claimed in order to satisfy description requirement, since lack of literal basis in disclosure for limitation that decomposition step of claims be "conducted in the absence of a catalyst" thus does not establish prima facie case for lack of descriptive support, and since it cannot be held that originally-filed disclosure would not have conveyed concept of effecting decomposition at elevated temperature in absence of catalyst.

2. Practice and procedure in Patent and Trademark Office — Reissue — Broader claims sought (§110.1313)

Claims in reissue application for method of determining nitrogen content of sample are overbroad under 35 USC 251, since application was filed more than two years after grant of original patent, since any claim which does not contain negative limitation expressly excluding presence of catalyst in decomposition step of method is broader than original claims, and since claims in question do not accomplish such exclusion by reciting phrase "consisting essentially of" in characterizing decomposition step.

Particular patents — Chemical — Nitrogen detection

4,018,562, Parks and Marietta, chemiluminescent nitrogen detection apparatus and method, claims 81-93 in application for reissue rejected.

Appeal from final rejection of claims in application for reissue of patent (Jill Johnston, primary examiner).

Application of Robert E. Parks and Robert L. Marietta, serial no. 708,810, filed May 31, 1991, continuation of serial no. 340,540, filed April 18, 1989 and abandoned, for reissue of patent no. 4,018,562, granted April 19, 1977 on application serial no. 625,510, filed Oct. 24, 1975 (chemiluminescent nitrogen detection apparatus and method). From final rejection of all claims in application, applicants appeal. Rejection of claims 1-10, 20-22, 55-80, and 94-106 reversed; rejection of claims 81-93 affirmed.

Before Calvert, vice chairman, and Steiner and Tarring, examiners-in-chief.

Steiner, examiner-in-chief.

This is an appeal from the final rejection of claims 1 through 10, 20 through 22 and 55 through 106, all the claims in this application for reissue of Patent No. 4,018,562 (the '562 patent).

THE INVENTION

The claimed invention is a method for determining the nitrogen content of a sample comprising manipulative steps which include decomposing the sample in an oxygen/inert gas atmosphere at an elevated temperature to obtain nitric oxide and causing the generated nitric acid to undergo a chemiluminescent reaction with ozone.

Claims 1, 81 and 94 are illustrative and read as follows:

1. The method for determining the total chemically combined nitrogen content of a sample comprising the steps:

a. decomposing said sample in one step in the presence of an oxygen-rich atmosphere of oxygen and an inert gas and at a temperature sufficiently above 700°C. that substantially all of the chemically bound nitrogen is recovered as nitric oxide (NO), such decomposition being conducted in the absence of a catalyst,

b. causing the nitric oxide produced by such decomposition to undergo a chemiluminescent reaction with ozone, and

c. determining the magnitude of the chemiluminescent reaction to indicate the quantity of chemically combined nitrogen in said sample.

81. A method for determining the total chemically combined nitrogen content of a sample, said method comprising the steps of:

(a) decomposing said sample in one step, said decomposing step consisting essentially of decomposing said sample in the presence of an oxygen-rich atmosphere of oxygen and an inert gas and at a temperature sufficiently above 700°C that substantially all of the chemically bound nitrogen is recovered as nitric acid (NO);

(b) causing the nitric oxide produced by such decomposition to undergo a chemiluminescent reaction with ozone; and

(c) determining the magnitude of the chemiluminescent reaction to indicate the quantity of chemically combined nitrogen in said sample.

94. A method for determining the total chemically combined nitrogen content of a sample, said method comprising the steps of:

(a) decomposing said sample in one step in the presence of an oxygen-rich atmosphere of oxygen and an inert gas and at a temperature sufficiently above 700°C that substantially all of the chemically bound nitrogen is recovered as nitric oxide (NO) according to the formula:

$$R\text{-}N+O_2 \triangleright CO_2+H_2O+NO$$

(b) causing the nitric oxide produced by such decomposition to undergo a chemiluminescent reaction with ozone; and

(c) determining the magnitude of the chemiluminescent reaction to indicate the quantity of chemically combined nitrogen in said sample.

THE REJECTIONS

Claims 1 through 10, 20 through 22 and 55 through 80 stand rejected under the first paragraph of 35 U.S.C. 112 for lack of adequate descriptive support. Claims 81 through 106 stand rejected under 35 U.S.C. 251 in that they are broader than the originally patented claims.[1] In addition, all the

[1] The ultimate paragraph of 35 U.S.C. 251 reads as follows:

No reissued patent shall be granted enlarging the scope of the claims of the original patent unless applied for within two years from the grant of the original patent.

appealed claims stand rejected under 35 U.S.C. 251 for lack of the requisite "error."

The rejection under the first paragraph of 35 U.S.C. 112, the rejection of claims 94 through 106 under 35 U.S.C. 251 as broader than the original claims, and the rejection of all the appealed claims under 35 U.S.C. 251 for lack of the requisite "error" are reversed; the rejection of claims 81 through 93 under 35 U.S.C. 251 as broader than the original claims is affirmed.

OPINION

The Rejection of Claims 1 through 10, 20 through 22 and 55 through 80 under the first paragraph of 35 U.S.C. 112.

The initial burden of establishing a *prima facie* basis to deny patentability to a claimed invention on any ground is always upon the examiner. *In re Oetiker*, 977 F.2d 1443, 24 USPQ2d 1443 (Fed. Cir. 1992). In rejecting a claim under the first paragraph of 35 U.S.C. 112 for lack of adequate descriptive support, it is incumbent upon the examiner to establish that the originally-filed disclosure would not have reasonably conveyed to one having ordinary skill in the art that an appellant had possession of the now claimed subject matter. *Wang Laboratories, Inc. v. Toshiba Corp.*, 993 F.2d 858, 26 USPQ2d 1767 (Fed. Cir. 1993). Adequate description under the first paragraph of 35 U.S.C. 112 does not require *literal* support for the claimed invention. *In re Herschler*, 591 F.2d 693, 200 USPQ 711 (CCPA 1979); *In re Edwards*, 568 F.2d 1349, 196 USPQ 465 (CCPA 1978; *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976). Rather, it is sufficient if the originally-filed disclosure would have conveyed to one having ordinary skill in the art that an appellant had possession of the concept of what is claimed. *In re Anderson*, 471 F.2d 1237, 176 USPQ 331 (CCPA 1973).

[1] The examiner contends that the rejected claims lack adequate descriptive support because there is "no literal basis for the"[1] claim limitation "in the absence of a catalyst." Clearly, the observation of a lack of literal support does not, in and of itself, establish a *prima facie* case for lack of adequate descriptive support under the first paragraph of 35 U.S.C. 112. *In re Herschler, supra; In re Edwards, supra; In re Wertheim, supra.*

The examiner notes that in *Parks v. Fine*, 773 F.2d 1577, 227 USPQ 432 (Fed. Cir. 1985), involving the claimed subject matter, the limitation "in the absence of a catalyst" was considered material. Suffice it to say, no issue under the first paragraph of 35 U.S.C. 112 for lack of adequate descriptive support for the limitation "in the absence of a catalyst" was before the court.

We are not unmindful of the decision in *Ex parte Grasselli*, 231 USPQ 393 (Bd.App. 1983) *aff'd mem.*, 738 F.2d 453 (Fed. Cir. 1984), which involved claims to a process for the ammoxidation of propane or isobutane employing a catalyst "free of uranium and the combination of vanadium and phosphorus." Under the particular facts in that case, it was held that the negative limitation introduced new concepts in violation of the description requirement of the first paragraph of 35 U.S.C. 112, citing *In re Anderson, supra.* In the situation before us,[2] it cannot be said that the originally-filed disclosure would not have conveyed to one having ordinary skill in the art that appellants had possession of the *concept* of conducting the decomposition step generating nitric acid in the absence of a catalyst. See, for example, column 5 of the '562 patent, first paragraph, wherein FIG. 4 is discussed. Pyrolysis temperatures of between 600°C and 700°C, and above 700°C were employed to achieve conversion of chemically bound nitrogen to nitric oxide. Smooth conversion was obtained above 700°C, while the optimum conversion was found to occur above 900°C. Throughout the discussion which would seem to cry out for a catalyst if one were used, no mention is made of a catalyst.[4]

Moreover, according to two declarations by Wentworth, a professor of chemistry at the University of Houston, whose expertise in this particular art has not been challenged, one having ordinary skill in the art would have recognized that the reaction generating nitric oxide, according to the equation disclosed in the '562 patent, is conducted without a catalyst. See *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111 (Fed. Cir. 1991); *In re Lemin*, 364 F.2d 864, 150 USPQ 546 (CCPA 1966). Thus, it cannot be said that the originally-filed disclosure would not have conveyed to one having ordinary skill in the art the concept of effecting decomposition at an elevated temperature in the absence of a catalyst. *In re Anderson, supra.*

Accordingly, the examiner's rejection of claims 1 through 10, 20 through 22 and 55 through 80 under the first paragraph of 35 U.S.C. 112 for lack of adequate descriptive support is reversed.

The Rejection of Claims 81 through 106 under 35 U.S.C. 251 as Broader than the Original Claims.

We initially observe that on page 6 of the Brief,

> appellants agree that any claim in the reissue application that does not contain a limitation that *means* "in the absence of a catalyst" is broader than original claims 1-10 and hence unpatentable under 35 USC 251 (appellants' emphasis).

Claims 81 through 106 do not contain a negative limitation which expressly precludes the presence of a catalyst. However, appellants contend that claims 81 through 93 exclude the presence of a catalyst by virtue of the phrase "consisting essentially of" in characterizing the decomposition step, and that claims 94 through 106 exclude the presence of a catalyst by virtue of the recited equation for the decomposition reaction, which equation does not reflect the presence of a catalyst.

[2] In our opinion, the phrase "consisting essentially of," as employed in claims 81 through 93, limits decomposition to a single step and, in that sense, is redundant since decomposition is performed "in one step." However, it is not apparent and appellants have not explained why the expression "consisting essentially of" excludes the presence of a catalyst during the recited decomposition step.[3] It would, therefore, appear that claims 81 through 93 are broader than original claims 1 through 10 and, hence, were properly rejected by the examiner under 35 U.S.C. 251. Accordingly, the examiner's rejection of claims 81 through 93 under 35 U.S.C. 251 is affirmed.

Claims 94 through 106 recite the decomposition reaction in a manner which, according to the Wentworth declarations, means that no catalyst was employed. *In re Lemin, supra.* Accordingly, claims 94 through 106 would not appear broader than original claims 1 through 10 and, hence, the examiner's rejection of claims 94 through 106 under 35 U.S.C. 251 is reversed.

The Rejection of the Appealed Claims Under 35 U.S.C. 251 for Lack of the Requisite Error.

This rejection is reversed essentially for the reasons advocated by appellants on appeal. We emphasize that the practice of submitting claims as a hedge against the possible invalidity of original claims has been judicially sanctioned. See, for example, *Hewlett-Packard Co. v. Bausch & Lomb, Inc.*, 882 F.2d 1556, 11 USPQ2d 1750 (Fed. Cir. 1989); *In re Altenpohl*, 500 F.2d 1151, 183 USPQ 38 (CCPA 1974); *In re Handel*, 312 F.2d 943, 136 USPQ 460 (CCPA 1963).

In summary, the examiner's rejection of claims 81 through 93 is affirmed; the rejection of claims 1 through 10, 20 through 22, 55 through 80 and 94 through 106 is reversed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR 1.136(a). See the final rule notice, 54 F.R. 29548 (July 13, 1989), 1105 O.G. 5 (August 1, 1989).

AFFIRMED-IN-PART.

[1] See page 4 of the Answer, second full paragraph, line 4, and page 7 thereof, last two lines.

[2] Whether the requirement for an adequate written description has been met is a question of fact and, hence, driven by the exigencies of each case. *Wang Laboratories, Inc. v. Toshiba Corp.*, 993 F.2d 858, 26 USPQ2d 1767 (Fed. Cir. 1993).

[3] Compare *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 229 USPQ 805, 812, note 6 (Fed. Cir. 1986).

[4] A "catalyst" normally functions to accelerate a particular reaction. See for example, Hawley, *Condensed Chemical Dictionary*, Tenth Edition, 1981, pp. 205 and 206, copies of which are enclosed for appellants' convenience and made of record.

U.S. Patent and Trademark Office
Board of Patent Appeals and Interferences

Ex parte Heymes

No. 93-1646

Decided November 9, 1993
Released January 4, 1994

PATENTS

1. Patentability/Validity — Obviousness — Relevant prior art — Particular inventions (§115.0903.03)

Patentability/Validity — Obviousness — Secondary considerations generally (§115.0907)

Application claims for chemical compounds were properly rejected as obvious under 35 USC 103, since claims are prima facie obvious in view of cited references, since record does not show that claimed compounds, which are intermediates to patented compounds having antibiotic properties, have no known utility other than as

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael A. Apicella et al.

Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

Docket No.: 875.001US2
Filed: October 13, 1998
Examiner: S. Devi

Serial No.: 09/077,572
Due Date: August 21, 2001
Group Art Unit: 1645

BOX AF
Commissioner for Patents
Washington, D.C. 20231

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X A return postcard.
X Notice of Appeal (1 Page).
X Check for Notice of Appeal fee of $310.00.

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SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938, Minneapolis, MN 55402 (612-373-6900)

By: _____
Atty: Ann S. Viksnins
Reg. No. 37,748

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(GENERAL)

S/N 09/077,572 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Michael A. Apicella et al.	Examiner: S. Devi	
Serial No.:	09/077,572	Group Art Unit: 1645	
Filed:	October 13, 1998	Docket: 875.001US2	
Title:	NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA		

NOTICE OF APPEAL FROM THE DECISION OF THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

BOX AF
Commissioner for Patents
Washington, D.C. 20231

In compliance with 37 C.F.R. § 1.191, Applicants hereby appeal to the Board of Patent Appeals and Interferences from the decision dated February 21, 2001, of the Examiner rejecting claims 22-26, 29, 32 and 33 of the above-identified patent application.

Our check in the amount of $310.00 is enclosed to pay the Notice of Appeal fee under 37 C.F.R. § 1.17(b).

We believe that no extension of time is necessary to respond to the Examiner's rejection, since Applicants filed their complete response within the two-month period from the date of mailing of the final Office Action. To date, Applicants have not received a further action from the Examiner, and are filing this Notice of Appeal to prevent possible abandonment.

If, in spite of the above explanation, a petition for extension and fees under 1.17(a) are deemed to be due, please consider this a request for extension, and charge any required fees to Deposit Account No. 19-0743.

Respectfully submitted,

MICHAEL A. APICELLA ET AL.

By Applicants' Attorneys,

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Date 17 August 2001 By _____
Ann S. Viksnins
Reg. No. 37,748

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Candis B. Buending
Name

_____
Signature

APPENDIX III

Art of Record and Other References

I. Art of Record

Karow *et al.*, *Journal of Bacteriology* 174:7407-7418 (1991)

Westphal *et al.*, *Methods Carbonydr. Chem.* 5:83-91 (1965)

JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 7407–7418
0021-9193/92/227407-12$02.00/0

Vol. 174, No. 22

The Lethal Phenotype Caused by Null Mutations in the *Escherichia coli htrB* Gene Is Suppressed by Mutations in the *accBC* Operon, Encoding Two Subunits of Acetyl Coenzyme A Carboxylase

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Received 17 July 1992/Accepted 18 September 1992

Insertion mutations in the *Escherichia coli htrB* gene result in the unique phenotype of not affecting growth at temperatures below 32.5°C but leading to a loss of viability at temperatures above this in rich media. When *htrB* bacteria growing in rich media were shifted to the nonpermissive temperature of 42°C, they continued to grow at a rate similar to that at 30°C but they produced phospholipids at the rate required for growth at 42°C. This led to the accumulation of more than twice as much phospholipid per milligram of protein compared with that in wild-type bacteria. Consistent with HtrB playing a role in phospholipid biosynthesis, one complementation group of spontaneously arising mutations that suppressed *htrB*-induced lethality were mapped to the *accBC* operon. This operon codes for the biotin carboxyl carrier protein and biotin carboxylase subunits of the acetyl coenzyme A carboxylase enzyme complex, which catalyzes the first step in fatty acid biosynthesis. Four suppressor mutations mapped to this operon. Two alleles were identified as mutations in the *accC* gene, the third allele was identified as a mutation in the *accB* gene, and the fourth allele was shown to be an insertion of an IS*1* transposable element in the promoter region of the operon, resulting in reduced transcription. The suppressor mutations caused a decrease in the rate of phospholipid biosynthesis, restoring the balance between the biosynthesis of phospholipids and growth rate, thus enabling *htrB* bacteria to grow at high temperatures.

During a screen for new *Escherichia coli* heat shock genes, two insertion mutations in the *htrB* gene were isolated. Bacteria carrying either of these two mutations grow in a completely wild-type manner at temperatures below 32.5°C but are inviable at higher temperatures in rich media (23). Although *htrB* was later shown not to be a heat shock gene (24), its unique temperature requirement is intriguing because, for the most part, *E. coli* cells growing between 21 and 37°C show very few temperature-dependent adaptive responses (20). One of the changes that occurs is an alteration in the composition of lipids that is required for the maintenance of membrane fluidity (30). Originally, we disregarded the possibility that HtrB may directly affect membrane structure because known mutants unable to correctly alter their lipid composition are viable at all temperatures (9, 40). Rather, on the basis of the similarity of the morphology of *htrB* bacteria grown at nonpermissive temperatures to that of cell wall biosynthesis mutants (5), we proposed that HtrB was involved in cell wall biosynthesis (23). However, further studies have led us to conclude that HtrB most likely does play a role in membrane structure.

This conclusion originated from the study of a multicopy suppressor of *htrB*, *msbB*. These multicopy suppressors are genes that when cloned onto multicopy plasmids rescue the Ts⁻ phenotype of *htrB*. The protein encoded by *msbB* may serve a role similar to that of HtrB, since the MsbB protein sequence is similar to that of HtrR (25). In addition, null mutations in either the *msbB* or *htrB* gene result in a similar and unique phenotype, namely, the ability to grow on fourfold higher concentrations of deoxycholate than the wild-type bacteria (25). The increased resistance to deoxycholate most likely indicates that *htrB* and *msbB* bacteria have alterations affecting membrane structure, possibly the lipopolysaccharide (LPS) layer.

In general, mutations that affect the LPS layer alter the resistance of bacteria to hydrophobic compounds. Although most known LPS mutants are hypersensitive to hydrophobic molecules (35), there are a few mutants which exhibit increased resistance to hydrophobic molecules. The best studied of these is a mutant of *Salmonella typhimurium*, a *pmrA* mutant (41). This mutant exhibits an increased resistance to the hydrophobic drug polymyxin B. The increase in resistance has been shown to be associated with a decrease in the positive charge of a portion of the LPS molecules, thus reducing the number of binding sites for the negatively charged polymyxin B (42). A similar type of change in LPS structure may lead to the increased resistance to deoxycholate of *htrB* and *msbB* bacteria. For example, a decrease in the amount of LPS molecules with negatively charged phosphoethanolamine residues could lead to fewer binding sites for the positively charged deoxycholate molecules.

Another indication that HtrB affects the membrane structure is that low levels of cationic detergents suppress its Ts⁻ phenotype (25). The cationic detergents may act by altering the interactions between the LPS molecules and divalent cations or polyamines. The addition of Ca^{2+} or Mg^{2+}

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TABLE 1. Strains

Strain	Relevant characteristic(s)	Reference or source
W3110	Wild type	Our collection
B178	W3110 *galE*	14
MLK53	W3110 *htrB1*::Tn*10*	23
MLK777	B178 *htrB1*::Tn*10* *zhb-43*::Tn*10*-Kan^r	This work
MLK993	MLK53 Ts$^+$1031 *zhb-43*::Tn*10*-Kan^r	This work
MLK1000	MLK53 Ts$^+$1123 *zhb-43*::Tn*10*-Kan^r	This work
MLK995	MLK53 Ts$^+$1043-1 *zhb-43*::Tn*10*-Kan^r	This work
MLK994	MLK53 Ts$^+$1043-6 *zhb-43*::Tn*10*-Kan^r	This work
MLK519	B178 *htrB1*::Tn*10* Ts$^+$1031(λ)	This work
MLK1067	W3110 *msbB*::Ωcam	25
MLK986	MLK53 *msbB*::Ωcam	25
MLK1066	MLK986 Ts$^+$1043-1 *zhb-43*::Tn*10*-Kan^r	This work
MLK1067	MLK986 Ts$^+$1043-6 *zhb-43*::Tn*10*-Kan^r	This work
DH5α	*recA1*	Bethesda Research Laboratories

reverses this rescue, possibly by competing for the same sites on the LPS molecules (25). These results have led us to propose that HtrB affects outer membrane structure and function (25).

In an attempt to further understand the role of HtrB in bacterial physiology, we have undertaken the study of a second type of *htrB* suppressor: single-copy, spontaneously arising, extragenic suppressor mutations. Presumably, these mutations directly or indirectly alter functions that are affected by the lack of HtrB. By mapping these suppressor mutations and identifying the genes that encode them, we hoped to clarify the role that HtrB plays in *E. coli* physiology. Consistent with the proposal that HtrB plays a role in membrane structure and function, we report here that one complementation group of such suppressors affects the biosynthesis of phospholipids and that HtrB may play a role in the coupling of phospholipid biosynthesis and growth rate.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this study are shown in Table 1. Early work with the suppressor mutations was done in the B178 background. B178 carries a *galE* mutation which blocks the mucoidy associated with wild-type bacteria (14). After the discovery that HtrB itself may affect membrane function, all mutations were moved into the wild-type W3110 background. All experiments presented here were performed using this background strain, unless otherwise indicated. Bacteria were grown in Luria-Bertani (LB) medium prepared as described previously (23). L agar is LB medium with 1% agar. Antibiotics were added when needed, at the following final concentrations: ampicillin, 50 μg/ml; spectinomycin, 50 μg/ml; tetracycline, 10 μg/ml; chloramphenicol, 12 μg/ml; and kanamycin, 50 μg/ml.

Cell growth analysis. Bacterial growth, viability experiments, and photography were performed as described previously (23).

Plasmids. The pKS-1031 and pKS-1031-2 plasmids carry the 6-kb *Bam*HI fragment of λ transducing phage 6G3 (λ529) from the library made by Kohara et al. (26), cloned in both orientations into the *Bam*HI site of the pBluescript-KS plasmid (Stratagene). In pKS-1031, the fragment is oriented such that the T7 promoter on the plasmid is located 5′ to all of the open reading frames encoded on the fragment. The pBK2 plasmid was made by partially digesting pKS-1031 with *Kpn*I, digesting it to completion with *Bam*HI, isolating the 4-kb fragment from a low-melting-point agarose gel (FMC), and ligating the fragment to *Bam*HI-*Kpn*I-digested pBluescript-SK plasmid DNA. The p3/49 and p3/58 plasmids and the p2/53 plasmid are deletion derivatives of pKS-1031 and pKS-1031-2, respectively. These deletions were made according to the DNase I method of Hong (19). After partial DNase I digestion, the DNA was digested with *Eco*RI, ligated, and digested with *Pst*I to enrich for plasmids with deletions. The pE1 and pEK plasmids are the 900-bp *Eco*RV and *Eco*RV-*Kpn*I fragments of pKS-1031 cloned into the *Eco*RV and *Eco*RV-*Kpn*I sites of pBluescript-KS, respectively. The pGB-*accB* plasmid is the 2.3-kb *Eco*RV fragment from pKS-1031 cloned into the *Sma*I site of pGB2 (8). The pLac-*accC* plasmid was made by first digesting pKS-1031 with *Kpn*I and then partially digesting it with *Eco*RV. The 1.85-kb fragment was isolated from low-melting-point agarose and ligated with *Eco*RV- and *Kpn*I-digested pBluescript-KS plasmid DNA.

Genetic manipulations. P1-mediated transductions were performed as described by Miller (31).

Isolation of mini-Tn*10*-Kan^r elements linked to the cold-sensitive (Cs$^-$) suppressor mutations was accomplished by P1 transduction of a library of mini-Tn*10*-Kan^r insertions (45) into the suppressor strains, selecting simultaneously for Kan^r and colony formation at 30°C. The Cs$^+$ Kan^r colonies were then restreaked at 30 and 42°C. Normal growth at 30°C but inviability at 42°C indicated that the wild-type copy of the suppressor mutation was cotransduced with the mini-Tn*10*-Kan^r marker.

To determine complementation of the Ts$^+$1031 Cs$^-$ phenotype with the Kohara et al. λ clones (26), an aliquot of each clone was used to infect a fresh culture of *htrB* Ts$^+$1031(λ) (MLK519) and the bacteria were plated at 30°C. Colonies that grew were restreaked at 30 and 42°C to identify which phage clones complemented the growth defect.

Cloning and mapping of the *zhb-43*::Tn*10*-Kan^r marker. The *zhb-43*::Tn*10*-Kan^r marker was transduced into strain CG1151 (MC1040-2 carrying the Cam^R vector Mu d5005 [15]). A library of mini-Mu clones was made as described by Groisman and Casadaban (15) and plated on W3110(Mu) (MLK47). Clones which carry the mini-Tn*10*-Kan^r marker were isolated by selecting simultaneously for Kan^r and Cam^r, and one of them was used to probe the Kohara library of λ clones (26) by the techniques previously described (23).

PCR. Polymerase chain reactions (PCRs) were carried out by the method of Innis and Gelfand (21). The two primers used to amplify the coding region of *accB* were 5′-GCAATC

TCGCCGCCGGTTGGC-3' and 5'-GAACGGTCGCCGGA GCGGCT-3'. The primers used to amplify the promoter region of the *accBC* operon were 5'-CGACCTCGTCCTCCC TGACG-3' and 5'-GAACGGTCGCCGGAGCGGCT-3'.

DNA sequencing. Sequencing was done with Sequenase (version 2.1) as described by the manufacturer (United States Biochemical). PCR products were sequenced by the snap-cooling method of Kusukawa et al. (28). The 5'-CGACCTCGTCCTCCCTGACG-3' primer, used to make the PCR products, was also used to sequence across one IS*1* junction. The other junction was sequenced with a primer homologous to the IS*1* element, 5'-CCATCATACACT AAATCAG-3'.

Northern blot analysis. Isolation of RNA and Northern (RNA) blot analysis were performed as described previously (24). The *accBC* probe was the *Hin*dIII-*Pst*I DNA fragment internal to *accBC* and was labeled as described previously (24). To control for even loading of the RNA samples, the blot was stained with methylene blue after the hybridization procedure (18).

Western blot analysis. Western blots (immunoblots) were carried out as described by Ang and Georgopoulos (3). Streptavidin conjugated with alkaline phosphatase (Bethesda Research Laboratories) was used to detect the biotinylated BCCP with the chemiluminescence detection kit Western-light, from Tropix.

Fatty acid analysis. Bacterial cultures were first grown at their permissive temperatures to mid-log phase in LB medium, diluted into 50 or 100 ml of the same medium to an optical density at 595 nm (OD_{595}) of 0.05, and grown at 30 or 42°C to an OD_{595} of 0.4. The bacteria were harvested by centrifugation, and after the bacteria were washed twice with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.9) or 10 mM $MgCl_2$, fatty acids were extracted by the method of Bligh and Dyer (6) as described by Ames (2). The phospholipid fraction was isolated by direct extraction of the washed bacteria. The LPS-enriched fraction was isolated from the monophase of this extraction, present in the insoluble pellet, as described by Galloway and Raetz (12). The pellet was hydrolyzed in 6 N HCl for 3 h at 100°C, and fatty acids were extracted by the method of Bligh and Dyer (6). Methyl esters of the fatty acids were made with BF_3 and analyzed by gas chromatography on Supelco SPB-1 fused silica (15 m by 0.25 mm with 1-μm film thickness), with helium as the carrier gas (10 lb/in^2) and a temperature program of 5°C/min, 150 to 250°C. Supelco bacterial fatty acid methyl ester CP mixture 4-7080 was used to determine retention times of the individual fatty acids.

Quantification of esterified fatty acids. Bacterial cultures were grown as described for fatty acid analysis. After pelleting by centrifugation, the bacteria were washed once with 10 mM $MgCl_2$, and the phospholipids were isolated by the method of Bligh and Dyer (6). Following lyophilization, the lipids were quantitated as hydroxamates by the method of Stern and Shapiro (38). Standard curves were obtained with L-α-phosphatidylcholine di-heptadecanoyl (Sigma). All phospholipid quantities are given using an average molecular weight of 700.

Quantification of 3-deoxy-D-manno-octulosonic acid. Bacterial cultures of 400 ml were grown as described for fatty acid analysis. After the bacteria were washed once with ice-cold 50 mM Tris (pH 7.9) and resuspended in the same buffer, they were passed twice through a French press. The suspension was centrifuged at 5,000 × *g* for 5 min to remove large debris, and the supernatant was centrifuged at 30,000 × *g* for 60 min at 4°C. The resulting pellet was used as the outer membrane fraction and was washed once with 50 mM Tris (pH 7.9) and once with 10 mM HEPES (pH 7.4) buffer. The remaining supernatant was centrifuged for 60 min at 175,000 × *g* at 4°C. The resulting pellet was used as the inner membrane fraction and was washed as described above. The inner and outer membrane fractions were resuspended in 50 μl of distilled H_2O and assayed for protein as described below and for 3-deoxy-D-manno-octulosonic acid by the method of Weissbach and Hurwitz (46). LPS purchased from Sigma was used as the standard.

Protein determination. Following the washing procedures described above, a portion of each culture was lysed in 0.5% sodium dodecyl sulfate–10 mM EDTA–10 mM Tris (pH 7.9) at room temperature or at 55°C for highly concentrated suspensions of bacteria. Protein concentrations were determined with the bicinchoninic acid protein assay reagent purchased from Pierce, with bovine serum albumin as the standard.

Determination of phospholipid biosynthesis rates. Bacteria were grown at their permissive temperatures to mid-log phase in LB medium and diluted to an OD_{595} equal to 0.05, 10 μCi of [1-^{14}C]acetate (NEN-Dupont) was added, and the 1-ml cultures were shifted to 42°C at time zero. Aliquots were taken at the appropriate times, and the phospholipids were extracted by the method of Bligh and Dyer (6). The chloroform-solubilized phospholipids were washed twice with 2 M KCl and once with distilled H_2O before scintillation counting.

RESULTS

Identification and cloning of the wild-type copies of the suppressor genes. Extragenic suppressors of the *htrB* insertion mutations arise spontaneously at a frequency of approximately 10^{-4} at the nonpermissive temperature of 42°C. Approximately one-third of these suppressor mutations show, to various degrees, a Cs$^-$ phenotype (i.e., slow or no growth at 30°C or below). Using this Cs$^-$ phenotype, complementation analysis with linked mini-Tn*10*-Kan^r markers (45) was performed to assign these suppressor mutations into several different complementation groups (data not shown). One of these classes consisted of two alleles, Ts$^+$1123 and Ts$^+$1031, that were extremely cold sensitive, being unable to form colonies at 30°C or below. Because of the tight Cs$^-$ phenotype of these two mutations, we were able to clone their corresponding wild-type genes by complementation.

The cloning of the wild-type genes in which the Ts$^+$1123 and Ts$^+$1031 mutations were located was done by first localizing a closely linked mini-Tn*10*-Kan^r element, *zhb-43*::Tn*10*-Kan^r. This was accomplished by cloning the *zhb-43*::Tn*10*-Kan^r marker into a mini-Mu plasmid (described in Materials and Methods) and using [α-^{32}P]dATP-labeled plasmid DNA to probe the overlapping λ clones of the *E. coli* genomic library made by Kohara et al. (26). The *zhb-43*::Tn*10*-Kan^r mini-Mu plasmid DNA hybridized to phage clones 6G3, 6G9, and 3C5 (λ529 to λ531), corresponding to the 71-min region on the *E. coli* chromosome. To identify the phage(s) that carries the intact wild-type gene, we infected *htrB* Ts$^+$1031(λ) (MLK519) bacteria with phage clones 3G10 to 4G11 (λ523 to λ535), covering a total of 70 kb of DNA on each side of the *zhb-43*::Tn*10*-Kan^r marker. Among those recombinant phages tested, only 21D3 and 6G3 (λ528 and λ529) complemented the Cs$^-$ phenotype of the Ts$^+$1031 mutation.

We further localized the complementation activity to a *Bam*HI fragment of approximately 6 kb that was isolated

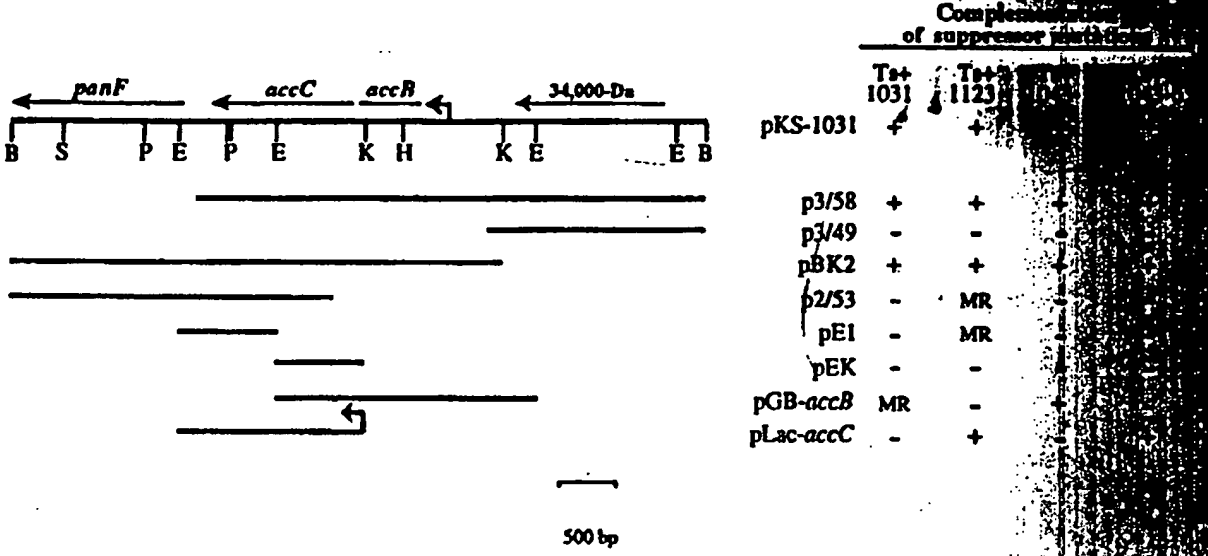

FIG. 1. Restriction map of the pKS-1031 plasmid and complementation of the suppressor mutations. The directions of transcription and open reading frames of the four genes encoded on pKS-1031 are indicated above the restriction map. Restriction sites are marked with abbreviated forms of the names of the restriction endonucleases: B, *Bam*HI; S, *Sal*I; P, *Pst*I; E, *Eco*RV; K, *Kpn*I; H, *Hin*dIII. Deletion derivatives and subclones are shown below the restriction map, the bars indicate the portion of pKS-1031 that is cloned in each case, and the name of each clone is shown to the right along with its ability to complement. The arrow above the pLac-*accC* clone represents the *lacZ* promoter encoded on the vector. Complementation of the suppressor mutations is indicated with a plus symbol, marker rescue of the mutation is indicated as MR, and noncomplementing clones are indicated with a minus symbol.

from λ6G3 and cloned into pBluescript-KS (pKS-1031). A combination of methods, including partial DNA sequencing, analysis of the proteins encoded on this fragment with the T7 polymerase-promoter system of Tabor and Richardson (39), and comparison of the restriction map of pKS-1031 to the published restriction maps of this region (1, 22, 29, 34, 43) (data not shown), identified this 6-kb clone as carrying the genes coding for pantothenate permease (*panF*), BCCP (*accB*), biotin carboxylase (*accC*), and a 34,000-Da protein of unknown function. Figure 1 shows the arrangement of these genes on this 6-kb fragment.

A set of deletion derivatives and subclones of this fragment were made to determine which of these genes was required for complementation of the Cs$^-$ phenotype of the Ts$^+$1031 and Ts$^+$1123 mutations. It was found that only deletion derivatives p3/58 and pBK2 were able to complement (Fig. 1). The *accBC* genes, coding for BCCP and biotin carboxylase, are the only genes common to both of these derivatives. These two genes have recently been shown to form an operon, with the promoter located 5′ to the *accB* gene (29) (Fig. 1).

Isolation of non-cold-sensitive suppressor alleles. To determine whether the extreme Cs$^-$ phenotype was invariably linked with the ability of these mutations to suppress *htrB*, we isolated new alleles without prior screening for a Cs$^-$ phenotype. To do this we transduced the *zhb-43*::Tn*10*-Kan^r marker into *htrB* mutant bacteria, and three independent isolates were grown overnight in liquid at 42°C to allow suppressor mutations to accumulate. The *zhb-43*::Tn*10*-Kan^r marker and any suppressor mutations linked to it were transduced back into *htrB* bacteria and identified by selecting simultaneously for Kan^r and colony formation at 42°C. Only one Ts$^+$ suppressor isolate from each of the three original cultures was characterized to ensure that each new suppressor was due to an independent mutational event. Two of three such suppressor mutations, Ts$^+$1043-1 and Ts$^+$1043-6, were shown to be linked to the *zhb-43*::Tn*10*-Kan^r marker by transduction, and both were mapped to the *accBC* operon by complementation studies (Fig. 1). Since both of these new suppressor strains formed colonies at 30°C, it appears that the extreme Cs$^-$ phenotype is not a prerequisite for suppression. However, both mutations affected bacterial growth at 30°C (Fig. 2A); *htrB* Ts$^+$1043-6 bacteria grew slightly more slowly than the wild type, and *htrB* Ts$^+$1043-1 bacteria grew more slowly still, with a rate approaching that of *htrB* Ts$^+$1123 bacteria, which did not form colonies at 30°C.

Suppression of the HtrB phenotypes. All four of the suppressor mutations restored the ability of *htrB* bacteria to grow at 42°C, albeit at a lower growth rate than that of wild-type bacteria (Fig. 2B). The altered morphology that accompanies the loss of viability of *htrB* bacteria was also suppressed in these strains. Photographs of isogenic wild-type bacteria, *htrB* mutant bacteria, and *htrB* Ts$^+$1043-6 bacteria are shown in Fig. 2C. The *htrB* bacteria formed their characteristic bulges, whereas *htrB* bacteria with the Ts$^+$1043-6 suppressor mutation exhibited a wild-type morphology. Although the suppressor mutations suppressed the lethal phenotype caused by *htrB*, they did not suppress the increased deoxycholate resistance exhibited by *htrB* bacteria at 30°C. Whereas the MIC of deoxycholate for wild-type bacteria was 2.5%, both *htrB* and the suppressor strains grew on L agar supplemented with 10% deoxycholate (data not shown).

To further pursue the question of which of the HtrB phenotypes were reversed by the suppressor mutations, we checked their effects on *htrB msbB* double-mutant bacteria. The *msbB* gene was originally isolated as a multicopy suppressor of *htrB* and subsequently shown to code for

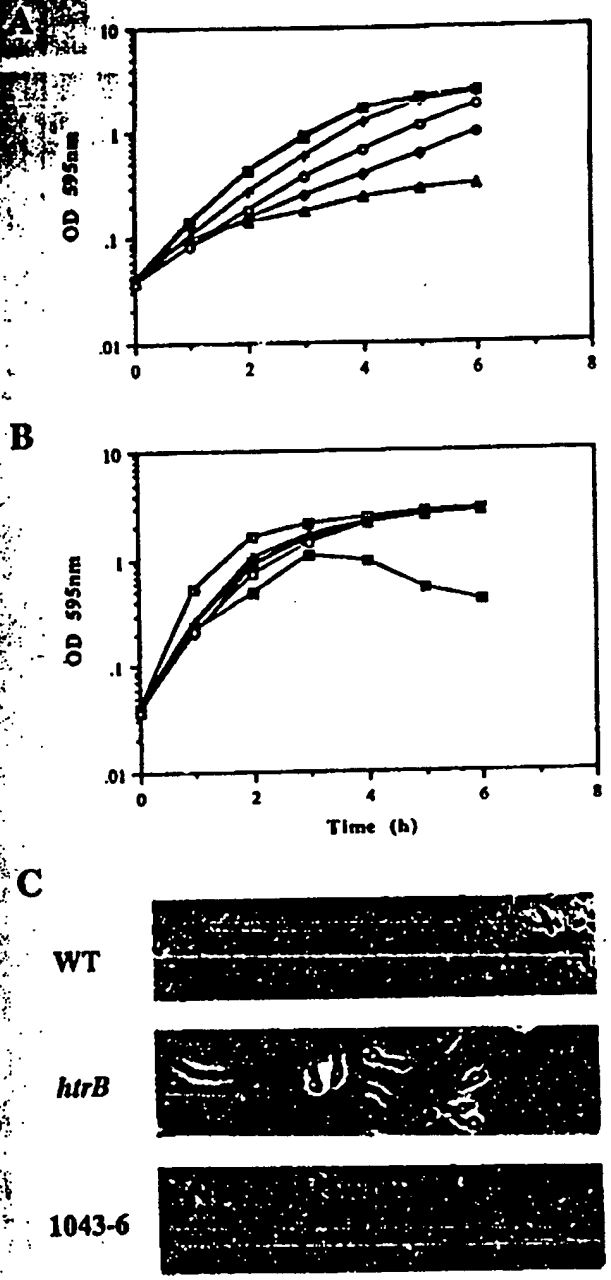

FIG. 2. Morphology and growth curves of wild-type, *htrB*, and various suppressor derivative bacteria. (A and B) Growth curves at 30°C (A) and 42°C (B) of isogenic wild-type (□), *htrB* (■), *htrB* Ts⁺1031 (△), *htrB* Ts⁺1123 (◇), *htrB* Ts⁺1043-1 (○), and *htrB* Ts⁺1043-6 (+) bacteria. (C) Photographs of wild-type (WT), *htrB*, and *htrB* Ts⁺1043-6 (1043-6) bacteria grown at 42°C from an OD_{595} of 0.05 for 2.75 h.

protein similar to HtrB (25). We previously concluded that MsbB plays a role similar to and possibly redundant with that of HtrB because *htrB msbB* double-mutant bacteria exhibit phenotypes at 30°C that are not associated with either of the single mutations, including a heterogeneous alteration in morphology (i.e., filamentous and fat, rather than shapen cells) and a growth rate that is lower than that of wild-type bacteria (25). The presence of the Ts⁺1043-1 or Ts⁺1043-6 mutation suppressed the morphological alterations of the *htrB msbB* bacteria, but the slow-growth phenotype was actually accentuated in the triple mutants (data not shown).

Molecular analysis of the suppressor mutations. To identify which of the two genes in the *accBC* operon were mutated in these suppressor strains, we cloned the two wild-type genes individually on separate plasmids and used them to map the location of the four suppressor mutations. The *accB* gene, coding for BCCP, was subcloned into the low-copy-number vector pGB2 (8) on a 2.3-kb *Eco*RV fragment (pGB-*accB*). This fragment was cloned into a low-copy-number vector because its cloning, or the cloning of any fragment carrying the *accB* gene alone, onto higher-copy-number vectors has been unsuccessful thus far. The biotin carboxylase gene, *accC*, was cloned onto a 1.85-kb *Kpn*I-*Eco*RV fragment under the control of the *lac* promoter of pBluescript-KS (pLac-*accC*). Figure 1 shows the relationship of these subclones to the full-length pKS-1031 clone and the locations of the coding regions for BCCP and biotin carboxylase.

When either pLac-*accC* or pGB-*accB* was transformed into *htrB* bacteria, the bacteria formed colonies at 42°C. This finding was surprising, since the pKS-1031 plasmid containing the entire *accBC* operon did not rescue *htrB* bacterial growth at 42°C (data not shown). This ability to rescue appears to be the result of a stoichiometric imbalance of these two enzyme subunits, altering the activity of the acetyl coenzyme A (acetyl-CoA) carboxylase enzyme complex in a manner analogous to that caused by the suppressor mutations themselves (see below).

Complementation of the Cs⁻ phenotype of the Ts⁺1031 and Ts⁺1123 mutations by pLac-*accC* and pGB-*accB* was determined by colony formation at 30°C. The pLac-*accC* plasmid complemented the Cs⁻ phenotype of the Ts⁺1123 mutation, indicating that this mutation mapped to the *accC* gene. We further localized Ts⁺1123, by marker rescue, to the 950-bp *Eco*RV fragment (pE1) in the region encoding the carboxy terminus of the biotin carboxylase protein (Fig. 1). The Cs⁻ phenotype of the Ts⁺1031 mutation was not complemented by either of the subclones, despite the fact that it was complemented by the full-length pKS-1031 plasmid (Fig. 1). However, the pGB-*accB* plasmid rescued *htrB* Ts⁺1031 mutant bacteria by recombination, indicating that the Ts⁺1031 mutation was located within this fragment. The inability of both pLac-*accC* and pGB-*accB* to complement the Cs⁻ phenotype of Ts⁺1031 could indicate that this mutation exerts a polar effect on the expression of the *accB* gene or affects the promoter region of the *accBC* operon. To differentiate between these two possibilities, this mutation was characterized further, as described below.

Because the Ts⁺1043-1 and Ts⁺1043-6 suppressor mutations did not exhibit an extreme Cs⁻ phenotype, one way to map them would have been to assess complementation by the reappearance of the Ts⁻ phenotype of *htrB*. However, the above-mentioned ability of either pLac-*accC* or pGB-*accB* to rescue *htrB* at 42°C made this strategy impossible. Fortuitously, the presence of either pLac-*accC* or pGB-*accB* did not fully reverse the morphological alterations exhibited by *htrB msbB* double-mutant bacteria, so this double-mutant background was used to map the Ts⁺1043-1 and Ts⁺1043-6 mutations. When *htrB msbB* Ts⁺1043-6 triple-mutant bacteria were transformed with the pLac-*accC* plasmid, the

resulting bacterial morphology was identical to that exhibited by the unsuppressed *htrB msbB* double mutant, indicating that Ts⁺1043-6 most likely was a mutation in the *accC* gene (Fig. 1). The opposite result was obtained with the *htrB msbB* Ts⁺1043-1 triple-mutant bacteria. The presence of the pGB-*accB* plasmid resulted in the appearance of filamentous cells, a phenotype exhibited by *htrB msbB* double-mutant bacteria carrying pGB-*accB*, indicating that Ts⁺1043-1 was most likely a mutation in the *accB* gene (Fig. 1).

Analysis of the Ts⁺1031 mutation. To determine whether the Ts⁺1031 mutation was a polar mutation in the *accB* gene or a promoter mutation, we used PCR to amplify the *accB* gene from genomic DNA isolated either from bacteria carrying the Ts⁺1031 mutation or from the isogenic wild-type strain. We first amplified and sequenced the coding region of the *accB* gene and found that there were no changes in the Ts⁺1031 DNA sequence. We then amplified the promoter region of the operon and found that the PCR product made from the DNA of the Ts⁺1031 mutant was approximately 750 bp longer than the corresponding PCR product made from wild-type DNA (data not shown). The sequencing of this PCR fragment showed that there was an IS*1* element inserted 215 bp upstream of the translational start codon for BCCP. Li and Cronan (29) have recently located the transcriptional start site of the *accBC* operon to 296 bp upstream of the *accB* coding region. Thus, the Ts⁺1031 mutation was an IS*1* element inserted within this unusually long, 5′-untranslated leader region (Fig. 3A). The *accBC* promoter has previously been shown to be located in a region of bent DNA (27, 29, 32, 34); the IS*1* element has inserted at one end of this bent DNA region. Like most IS*1* insertion events (11), a 9-bp direct repeat was created in the *accBC* DNA (Fig. 3A).

IS*1* elements have been shown to exert polar effects on transcription, as well as create new promoters at their site of insertion (11). These promoters are created by fusing a preexisting −35 promoter recognition sequence, within the IS*1* element, to potential −10 promoter recognition sequences in the genome. In this case it is likely that transcription from the *accBC* promoter terminated within the element and that the small quantity of residual transcription seen was due to initiation at a newly created promoter, much weaker than the *accBC* promoter, as illustrated in Fig. 3A. The activity of this promoter was low probably because the spacing between the putative −10 and −35 regions is 6 bp shorter than the average spacing between −10 and −35 regions (33).

Northern blot analysis was performed to determine the effect of the IS*1* element insertion on the transcription of the operon. To determine whether any of the other suppressor mutations affect the expression of the operon, we included RNA from the other suppressor strains, as well as *htrB* and wild-type bacteria, grown at either 30 or 42°C. As shown in Fig. 3B, only the Ts⁺1031 mutation had a substantial effect on *accBC* expression; the insertion of the IS*1* element was found to greatly reduce the transcription of this operon at both 30 and 42°C.

One would expect that such a large decrease in the amount of mRNA would be reflected by the amount of BCCP and biotin carboxylase protein present in the cell. Using streptavidin conjugated to alkaline phosphatase and a chemiluminescent substrate, biotinylated BCCP was detected on Western blots. As shown in Fig. 3C, the quantity of biotinylated BCCP was indeed reduced at 30°C, but surprisingly, at 42°C the reduction was not as much as would be expected considering the results obtained from the Northern blot experiment.

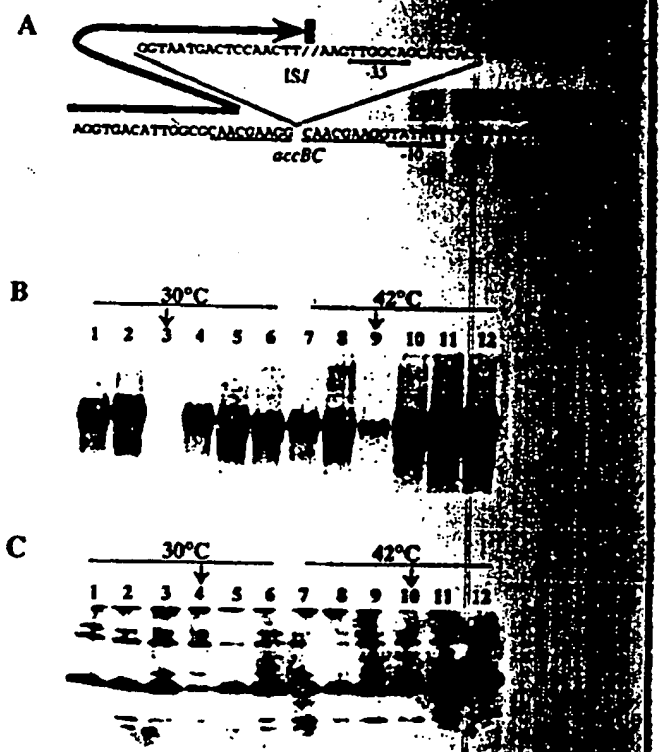

FIG. 3. Effects of the IS*1* transposable element insertion mutation, Ts⁺1031, on the expression of the *accBC* operon. (A) Model of the mechanism by which the insertion of the IS*1* element decreases the transcription of the *accBC* operon. The IS*1* element is indicated above the *accBC* DNA sequence, and the exact point of insertion in the *accBC* DNA is indicated by a gap in the sequence. The boldface arrow, initiating from the left, represents transcription from the *accBC* promoter. The bar at which this arrow ends indicates transcriptional termination within the IS*1* element. Potential −35 and −10 RNA polymerase recognition signals are marked. Thin lines below the *accBC* sequence indicate the 9-bp repeat created by the insertion of the IS*1* element. The small arrow to the right, above the *accBC* DNA sequence, represents the lower level of transcription initiated at this putative new promoter. (B) Northern blot of RNA isolated from wild-type (lanes 1 and 7), *htrB* (lanes 2 and 8), *htrB* Ts⁺1031 (lanes 3 and 9), *htrB* Ts⁺1123 (lanes 4 and 10), *htrB* Ts⁺1043-1 (lanes 5 and 11), and *htrB* Ts⁺1043-6 (lanes 6 and 12) bacteria grown at 30 or 42°C. (C) Western blot analysis of biotinylated BCCP protein from wild-type (lanes 1 and 7), *htrB* (lanes 2 and 8), *htrB* Ts⁺1123 (lanes 3 and 9), *htrB* Ts⁺1031 (lanes 4 and 10), *htrB* Ts⁺1043-1 (lanes 5 and 11), and *htrB* Ts⁺1043-6 (lanes 6 and 12) bacteria grown at 30 or 42°C. The arrows above the lanes serve to highlight the *htrB* Ts⁺1031 results. The bar to the right of the Western blot indicates the position of the BCCP protein.

Fatty acid analysis of *htrB* and the suppressor mutations. The only other known mutation of the *accBC* operon is *fabE* (16). This mutation results in a Ts⁻ phenotype and has recently been shown to be a point mutation in the *accB* gene near the region encoding the biotin attachment site of BCCP (29). When *fabE* or *fabD* Ts⁻ mutants (FabD catalyzes the second step in fatty acid biosynthesis) are grown at semipermissive temperatures, their fatty acid compositions are altered (16, 17). This alteration reflects the use of most of the

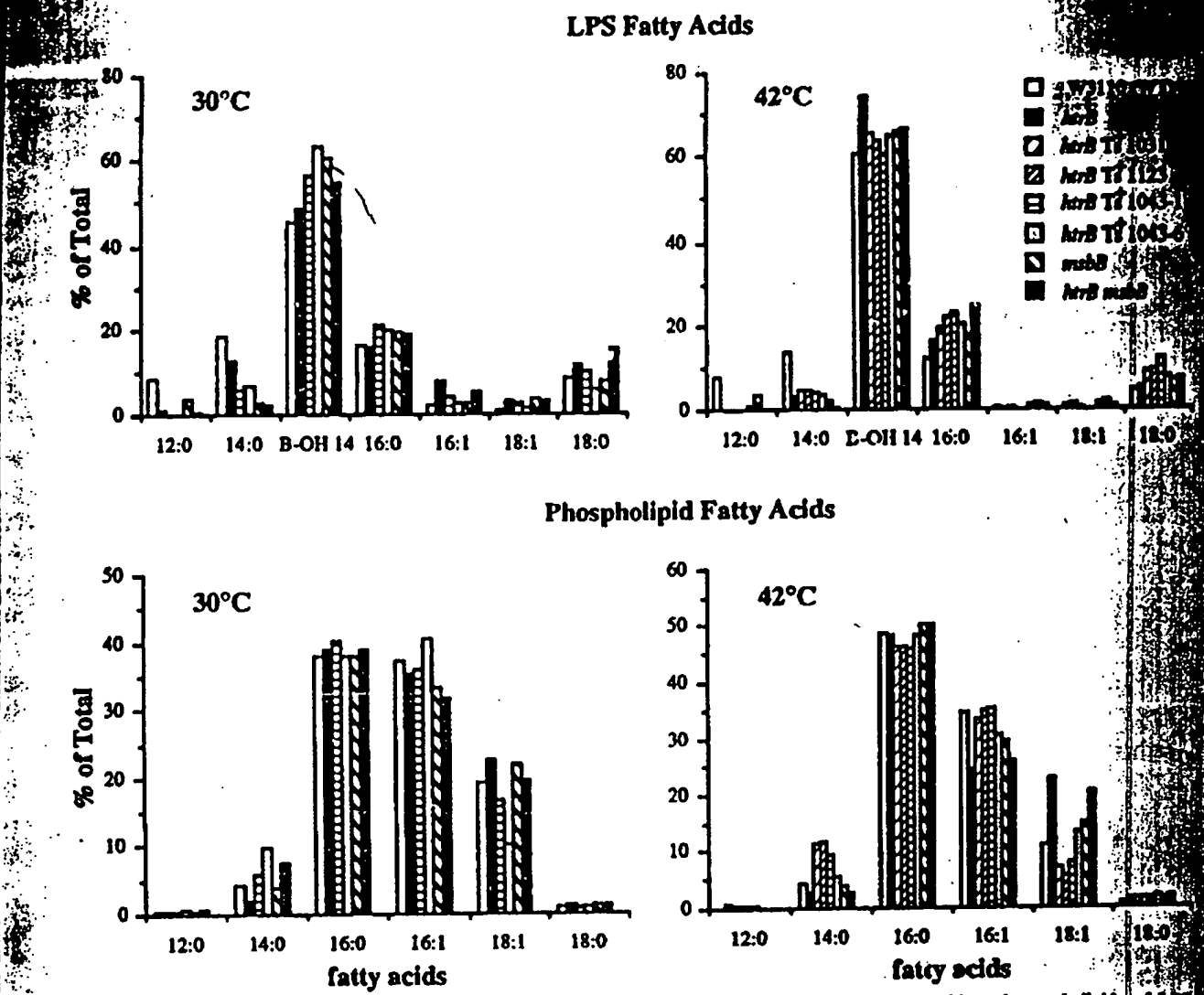

FIG. 4. Fatty acid compositions. Shown are graphical representations of the percent fatty acid composition of phospholipid and LPS fractions from wild-type (WT), *htrB*, *htrB* suppressor strains, *msbB*, and *msbB htrB* bacteria. β-Hydroxymyristic acid is abbreviated as β-OH 14. The phospholipid and LPS fatty acid percentages at 30 and 42°C for wild-type, *htrB*, *msbB*, and *htrB msbB* bacteria are the averages from four independent experiments. The fatty acid percentages for phospholipid and LPS fractions at 42°C for the suppressor strains are the averages from two independent experiments. The fatty acid percentages for phospholipid and LPS fractions at 30°C for the suppressor strains are from a single experiment.

residual enzymatic activity to form β-hydroxymyristic acid, the major fatty acid of LPS.

Because of the observed effects of the *fabE* and *fabD* mutations, we reasoned that the *accBC* suppressor mutations may also alter fatty acid composition and that this might compensate for the effects caused by the lack of HtrB. To determine whether this was the case, we analyzed the fatty acid compositions of both phospholipid- and LPS-enriched fractions isolated from *htrB* bacteria, the suppressor strains, and isogenic wild-type bacteria. It was found that both the LPS and phospholipid fatty acid compositions of *htrB* bacteria were altered (Fig. 4). The LPS fatty acids from *htrB* bacteria grown at either 30 or 42°C exhibited reproducible reductions in lauric acid (12:0) and myristic acid (14:0). At 30°C, there was a slight increase in palmitoleic acid (16:1), and at 42°C, there was an increase in palmitic acid (16:0) and β-hydroxymyristic acid (compare the open and black bars in Fig. 4). Such increases may compensate for the lack of the smaller fatty acids. Rather than reversing these changes, the suppressor mutations actually accentuated the observed decreases in myristic acid (14:0) at 30°C, suggesting that these changes were probably not the cause of *htrB* lethality.

Although the *htrB* mutation had only a slight effect on phospholipid fatty acid composition at 30°C, at 42°C the ratio of the two unsaturated fatty acids, palmitoleic acid (16:1) and *cis*-vaccenic acid (18:1), was considerably altered (compare open and black bars in Fig. 4). The 18:1/16:1 ratio for *htrB* bacteria was 0.93, whereas wild-type bacteria had a ratio of

0.32. However, the total percentage of unsaturated fatty acids remained similar to that for the wild type, 45.8% for wild-type bacteria and 47.3% for *htrB* bacteria. All four suppressors reversed the effect on the 18:1/16:1 ratio, and in the case of the Ts$^+$1031, Ts$^+$1123, and Ts$^+$1043-1 suppressor mutations, there was a slight overcompensation, resulting in 18:1/16:1 ratios between 0.16 and 0.22. Although the ability of the suppressor mutations to reverse the alterations in fatty acid composition indicates that these changes were linked to *htrB* lethality, it is unlikely that these changes were the direct cause of *htrB* lethality, since they are similar to and not as extreme as those changes caused by the Vtr mutation of the *fabF* gene, which has no effect on bacterial viability (9).

In an attempt to further define which of the fatty acid composition changes were associated with *htrB* lethality, we also determined the effects of a null mutation in the *msbB* gene and the effects of an *htrB msbB* double mutation. Because HtrB and MsbB appear to share similar functions but, unlike HtrB, MsbB is not required for growth under any condition tested (25), we reasoned that by comparing changes caused by the *msbB* null mutation with those caused by the *htrB* mutation we could determine which changes were associated with the nonlethal membrane alterations and which were associated with *htrB* lethality.

We found that the *msbB* mutation caused a qualitative alteration in LPS fatty acids similar to that seen with *htrB*. Thus, these changes were most likely associated with nonlethal changes in membrane structure. The *msbB* mutation resulted in a slight change in the phospholipid 18:1/16:1 ratio but not as much as that caused by the *htrB* mutation, a result consistent with the phospholipid fatty acid changes being associated with *htrB* lethality. The *htrB* and *msbB* changes in LPS fatty acids appear to be additive, since the *htrB msbB* double mutation resulted in an effect that was greater than that seen with either single mutation (Fig. 4). At 42°C the double mutation had an effect similar to that of *htrB* alone. This was an expected result, since in all other respects *htrB* has been shown to be epistatic to *msbB* at 42°C (25).

The quantity of phospholipids per milligram of protein. Although the results from the fatty acid analysis suggested that the changes in phospholipid fatty acid composition were associated with *htrB* lethality, no clear relationship between these changes and lethality could be discerned. However, during this analysis we noted an overall increase in the amount of fatty acids present in the phospholipid fraction per milligram of protein from *htrB* bacteria grown at 42°C. To determine whether *htrB* bacteria indeed had increased quantities of phospholipids, we used the hydroxamic quantification method of Stern and Shapiro (38). We standardized the amount of phospholipid to total cellular protein since the quantity of protein per OD_{595} unit of bacteria was not affected by the presence of the *htrB* mutation (data not shown). As shown in Table 2, at 42°C, *htrB* bacteria accumulate more than twice as much phospholipid per milligram of protein as wild-type bacteria do. In each case, the presence of the suppressor mutations inhibited this overproduction, leading to a phospholipid-to-protein ratio that was 94 to 123% of that seen with wild-type bacteria.

The ability of the Ts$^+$1043-1 and Ts$^+$1043-6 mutations to suppress the morphological phenotypes of the *htrB msbB* double mutant at 30°C suggests that this phenotype may also be caused by an increase in phospholipids. However, at 30°C the phospholipid levels for the *htrB msbB* double mutant and both of the single mutants were similar to that of wild-type bacteria (Table 2). Therefore, the morphological changes must be associated with another aspect of membrane biosynthesis that can also be suppressed by the Ts$^+$1043-1 and Ts$^+$1043-6 mutations. Consistent with the *msbB* mutation having no deleterious effects on bacterial growth, no increase in phospholipids at 30 or 42°C was observed (Table 2). Like all other phenotypes tested, *htrB msbB* double-mutant bacteria exhibited the same phenotypes at 42°C as *htrB* mutant bacteria, including the twofold overproduction of phospholipids (Table 2).

TABLE 2. Phospholipid levels for *htrB* and related bacteria at various temperatures

Growth temp (°C)[a]	Strain or relevant genotype	μg of phospholipid/mg of protein	% of wild type
42	W3110 (wild type)	139 ± 10	100
	htrB	326 ± 27	235
	htrB Ts$^+$1031	171 ± 2	123
	htrB Ts$^+$1123	153 ± 8	110
	htrB Ts$^+$1043-1	166 ± 10	119
	htrB Ts$^+$1043-6	130 ± 19	94
	msbB	138 ± 30	99
	htrB msbB	290 ± 30	209
30	W3110 (wild type)	163 ± 14	100
	htrB	146 ± 20	90
	msbB	127 ± 5	78
	htrB msbB	136 ± 9	83

[a] Bacteria were grown at the indicated temperature from an OD_{595} of 0.05 to an OD_{595} of 0.4. For more details, see Materials and Methods.

Because we have previously proposed that HtrB plays a role in outer membrane function (25), possibly affecting the LPS layer, we also determined the amount of LPS present in *htrB* bacteria. This determination was made by two methods. We first determined the amount of LPS fatty acids per milligram of protein by gas chromatography and found that there was no increase compared with the amount in wild-type bacteria. We also used the thiobarbituric acid method (46) to quantify the amount of 3-deoxy-D-manno-octulosonic acid residues present on LPS and found that there was no increase; wild-type bacteria had 546 ± 47 μg of LPS per mg of protein, and *htrB* bacteria had 591 ± 30 μg of LPS per mg of protein. However, we did find that there was an increase in the amount of LPS in the inner membrane fraction, accompanying a decrease in the amount of LPS in the outer membrane fraction. Whereas 83% of the LPS from wild-type bacteria sedimented with the outer membrane fraction, only 48% of the LPS from *htrB* bacteria sedimented with the outer membrane fraction. Determination of the amount of phospholipid in the two fractions indicated that both the inner and outer membranes contain increased quantities of phospholipids (data not shown). The shift of LPS to the inner membrane fraction was most likely a consequence of increased amounts of phospholipids in the outer membrane, thus decreasing its overall buoyant density, so that it fortuitously sedimented with the inner membrane fraction.

Determination of the rate of phospholipid biosynthesis. To establish the nature of the overproduction of phospholipids in *htrB* bacteria and the means by which the *accBC* mutations suppressed *htrB* lethality, we determined the rate of [1-^{14}C]acetate incorporation into phospholipids. As shown in Fig. 5, the rate of phospholipid biosynthesis was reduced by approximately 30 to 40% in all four suppressor strains compared with that of the wild type. This was an expected result, since all of the suppressor strains exhibited reduced

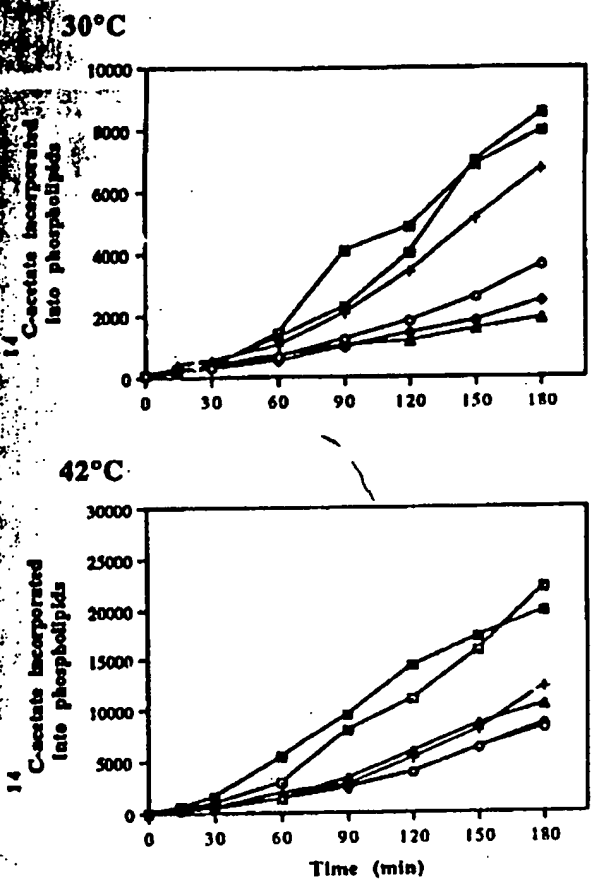

FIG. 5. Rate of phospholipid biosynthesis at 30 and 42°C. Shown is a graph of the [1-^{14}C]acetate incorporated into phospholipids as a function of time for wild-type (□) and *htrB* (■) bacteria and the Ts$^+$1031 (Δ), Ts$^+$1123 (◇), Ts$^+$1043-1 (○), and Ts$^+$1043-6 (+) suppressor strains.

rates of growth and the Ts$^+$1031 mutation caused a decrease in the transcription of the *accBC* operon.

As mentioned previously, Ts$^+$1031 and Ts$^+$1123 bacteria exhibit a Cs$^-$ phenotype and are unable to form colonies at 30°C. Both the Cs$^-$ and the slow-growth phenotypes are caused by the suppressor mutations and are not affected by the presence of the *htrB* mutation (data not shown). To determine whether the inability of Ts$^+$1031 and Ts$^+$1123 bacteria to form colonies at 30°C was due to a failure to synthesize phospholipids, we also measured the rate of phospholipid biosynthesis at 30°C. As shown in Fig. 5, both of these strains continued to synthesize phospholipids at a rate that was approximately 20 to 30% of that of wild type. Since these strains continued to synthesize phospholipids at 30°C, it is not obvious why Ts$^+$1031 and Ts$^+$1123 bacteria do not form colonies on L-agar plates even after prolonged incubation. The fact that Ts$^+$1043-1 bacteria exhibited a slightly a higher rate of fatty acid biosynthesis at 30°C than the two Cs$^-$ suppressor strains and formed small colonies at 30°C suggests that colony formation may require a threshold amount of phospholipid biosynthesis and that Ts$^+$1031 and Ts$^+$1123 bacteria do not exceed this threshold, but Ts$^+$1043-1 bacteria do.

Because the presence of either the pLac-*accC* or the pGB-*accB* plasmid allowed *htrB* bacteria to grow at 42°C, we also measured the rate of phospholipid biosynthesis for wild type and *htrB* strains carrying these plasmids. The presence of either of these plasmids led to a decrease in the rate of fatty acid biosynthesis in both wild-type and *htrB* bacteria. The presence of pLac-*accC* reduced the rate of fatty acid biosynthesis by approximately 65%, whereas pGB-*accB* reduced the rate by approximately 30%. The amount of reduction caused by these two plasmids directly reflected their abilities to allow *htrB* bacteria to grow at 42°C. Mutant *htrB* bacteria carrying pLac-*accC* formed almost wild-type-size colonies at 42°C, whereas those carrying pGB-*accB* formed only small colonies. The reduction in fatty acid biosynthesis caused by pGB-*accB* may be enough to permit colony formation of *htrB* bacteria at 42°C but not enough for rapid growth. As proposed above, the presence of either of these plasmids probably disrupts the stoichiometric balance of the subunits composing the acetyl-CoA carboxylase complex. This disruption and consequent reduction in fatty acid biosynthesis may also explain why the *accB* gene cannot be cloned alone on higher-copy-number plasmids. The increased amounts of BCCP may disrupt the complex to such a degree that fatty acid biosynthesis is dramatically affected.

At both 30 and 42°C, *htrB* bacteria exhibited wild-type rates of phospholipid biosynthesis. This indicates that the overproduction of phospholipids may not be the result of an increased rate of phospholipid biosynthesis, but rather it may reflect the uncoupling of the rate of phospholipid biosynthesis from the rate of growth. Consistent with this, when *htrB* bacteria were shifted to 42°C, they continued to grow at a rate similar to that at 30°C, as judged by OD_{595} (9) (Fig. 2). However, *htrB* bacteria synthesized phospholipids at the rate required for wild-type bacteria to grow at 42°C. Unlike the rate of phospholipid biosynthesis, the rate of protein synthesis remained coupled to the rate of growth at high temperatures (data not shown). Thus, the increase in phospholipid levels per milligram of protein was actually the consequence of protein biosynthesis remaining coupled to the reduced rate of growth and phospholipid biosynthetic rates increasing with temperature, independently of growth rate.

The uncoupling between growth and phospholipid biosynthesis rates is best exemplified by the ratio of incorporated [1-^{14}C]acetate counts into phospholipid per milligram of protein. For wild-type bacteria this ratio was 2,500 cpm/mg of protein. In contrast, the ratio for *htrB* bacteria was 16,400 cpm/mg of protein. The *accBC* suppressor mutations appear to reduce phospholipid biosynthesis so that growth and phospholipid biosynthesis are once again coupled. The corresponding ratios for Ts$^+$1031 and Ts$^+$1041-6 bacteria were 3,600 and 3,100 cpm/mg of protein, respectively, much reduced compared with that for *htrB* bacteria and similar to those for the wild type.

DISCUSSION

Four single-copy extragenic suppressors of *htrB* have been isolated and mapped to the *accBC* operon, which codes for BCCP and biotin carboxylase. These two proteins associate with a heterodimer of carboxyltransferase to form the acetyl-CoA carboxylase enzyme complex. This complex catalyzes the first step in fatty acid biosynthesis, namely, the carboxylation of acetyl-CoA to form malonyl-CoA. Two of the four suppressor mutations, Ts$^+$1123 and Ts$^+$1043-6,

were mapped to the *accC* gene, encoding biotin carboxylase, whereas the Ts⁺1043-1 allele was mapped to the *accB* gene, encoding BCCP. The fourth mutation, Ts⁺1031, was identified as an insertion of an *IS1* transposable element in the promoter region of the operon.

The effect of this IS*1* element was a large reduction of *accBC* operon transcription. We were surprised to find that such a large change in mRNA levels had only a small effect on the biotinylated BCCP levels at 42°C. One possible explanation for this is that the *accB* gene is under translational regulation and, hence, a low intracellular level of mRNA may have little effect on BCCP levels. The unusually long, 5′-untranslated region of this mRNA (29) could serve such a function. Alternatively, if a constant level of biotinylated BCCP is maintained in the cell (irrespective of the quantity of unbiotinylated BCCP), changes in mRNA levels may have little effect on the levels of biotinylated BCCP. One argument against this latter suggestion is that Fall and Vagelos (10) showed that most of the BCCP isolated from *E. coli* is in the biotinylated form. However, if unbiotinylated BCCP is unstable, either in vivo or during the isolation procedure, it would appear that a majority of the BCCP in the cell is biotinylated.

Whether the effects of the Ts⁺1031 mutation were mediated through a small decrease in both BCCP and biotin carboxylase levels or through a larger decrease in biotin carboxylase levels alone is not known at this time. At 30°C, the Ts⁺1031 mutation must affect the levels of both biotin carboxylase and BCCP, since neither pGB-*accB* nor pLac-*accC* complemented the Cs⁻ phenotype of Ts⁺1031; only the presence of the pKS-1031 plasmid, which carries both genes, resulted in growth at 30°C. It appears that suppression can be mediated through either of these gene products, since the Ts⁺1123 and Ts⁺1043-6 alleles were identified as mutations in the *accC* gene and Ts⁺1043-1 was identified as a mutation in *accB*. These results also suggest that suppression was mediated through the activity of acetyl-CoA carboxylase enzyme complex as a whole, rather than through any one of its individual components.

Because these suppressor mutations mapped to an operon whose products are involved in phospholipid biosynthesis, we studied the effects of the *htrB* null mutation on this process. When *htrB* bacteria are grown at temperatures above 33°C in rich media, they lose viability rapidly (23). This loss of viability is associated with a twofold increase in the amount of phospholipid per milligram of protein. The overproduction of phospholipids was the consequence of synthesizing phospholipids at a rate that appears to be in excess of that required to accommodate the reduced growth rate of *htrB* bacteria at 42°C. This uncoupling of phospholipid biosynthesis and growth rates appears to be integral part of *htrB* lethality at high temperatures, since the suppressor mutations most likely rescue by reducing the rate of phospholipid biosynthesis, thus matching the reduced rate of growth. The ability of either the pGB-*accB* or pLac-*accC* plasmid alone to rescue the lethal phenotype of *htrB* bacteria also appears to be the result of a decrease in the rate of phospholipid biosynthesis, presumably caused by an imbalance in the levels of the individual subunits of the complex.

The *htrB* mutation also affected the fatty acid composition of both LPS and phospholipids. At both 30 and 42°C, the LPS fatty acids from *htrB* bacteria were relatively depleted in lauric and myristic acid residues but relatively enriched in the larger fatty acid residues compared with those from the wild-type bacteria. The absence of MsbB, a protein with a sequence similar to that of HtrB, had a similar effect on LPS fatty acids. These changes in LPS fatty acid [illegible] cause of or reflect other changes in the LPS [illegible] result in the increased deoxycholate resistance exhibited by *htrB* and *msbB* bacteria (25). Consistent [illegible] suppressor mutations did not reverse the [illegible] cholate resistance, nor did they reverse the changes to LPS fatty acid composition. Thus, [illegible] oxycholate resistance and alterations in LPS fatty acid composition are the only phenotypes of *htrB* that we have been able to identify at permissive temperatures. These results suggest that LPS synthesis may be the primary target of the *htrB* mutation. Unlike the changes in phospholipid composition, the changes to LPS fatty acid composition were not accompanied by changes in the quantity of LPS. This could indicate that whereas phospholipid biosynthesis is limited by the rate of fatty acid biosynthesis, LPS biosynthesis is controlled at some other step in its biosynthesis.

In contrast to the changes to LPS, the phospholipid fatty acid composition changes exhibited by *htrB* bacteria at 42°C were reversed by the presence of the suppressor mutations. As mentioned previously, we do not believe that these changes cause *htrB* lethality, because the observed changes in fatty acid composition are reminiscent of those exhibited by bacteria with the Vtr mutation in the *fabF* gene, encoding β-ketoacyl-acyl carrier protein synthase II, which are able to grow at all temperatures (9). This enzyme elongates palmitoleic acid, forming *cis*-vaccenic acid. The activity of the wild-type enzyme itself is altered by temperature, such that as the temperature rises the activity of the enzyme decreases, leading to a relative decrease in *cis*-vaccenic acid levels at higher temperatures (13). The Vtr mutation results in an increase in the activity of this enzyme at all temperatures such that high levels of *cis*-vaccenic acid are synthesized independently of growth temperature (9). Similar to the Vtr mutation, the imbalance in phospholipid biosynthesis and growth rates caused by the lack of HtrB may somehow increase the activity of β-ketoacyl-acyl carrier protein synthase II such that *cis*-vaccenic acid levels are increased at the expense of palmitoleic acid levels. The decrease in smaller fatty acids in the LPS fractions from the suppressor strains could be the result of the decrease in fatty acid biosynthesis altering the balance between the utilization of the smaller fatty acids for elongation and their acylation to the lipid A portion of LPS.

Originally, we interpreted the formation of the bulges and filaments by *htrB* bacteria at the nonpermissive temperatures to be the consequence of changes in cell wall structure (23). Although the *htrB* mutant may have an altered cell wall structure, because of our finding of an excess of phospholipids in *htrB* bacteria we now suggest that the formation of the bulges may be more analogous to the formation of bulges caused by an overproduction of poly-β-hydroxybutyrate (37). Poly-β-hydroxybutyrate is a homopolymer of R-(−)-3-hydroxybutyrate produced as a storage molecule by a wide variety of bacteria. *E. coli* does not normally produce this polymer. However, when the genes encoding the biosynthetic enzymes for the polymer are expressed in *E. coli*, large quantities of it are produced, constituting up to 80% of its dry weight (37). Such high levels of poly-β-hydroxybutyrate can lead to altered morphologies, including the formation of bulges and filaments (37). *E. coli* may respond to the presence of excess phospholipids in *htrB* bacteria in the same manner in which it deals with the large quantities of this polymer, in both cases leading to the formation of bulges and filaments.

Taking all of our data together, it appears that the [illegible]

Ts⁻ phenotype of *htrB* is caused by the combination of an overall reduced growth rate and its uncoupling from the rate of phospholipid biosynthesis at high temperatures. Because *htrB* bacteria can grow at high temperatures under slow-growth conditions (23), such as in minimal media, we believe that the Ts⁻ phenotype exhibited by these bacteria is not wholly caused by the growth temperature but instead is a consequence of the increased growth rate at higher temperatures. When *htrB* bacteria are grown in rich media at temperatures above 33°C, they continue to grow at a rate that is similar to the rate at which they were growing at the permissive temperature of 30°C. The inability to adjust their growth rate in rich media at high temperatures suggests that in the absence of HtrB, the rate of some essential process is limited.

It is not clear why phospholipid biosynthesis does not remain coupled to the rate of growth in *htrB* bacteria. Growth rate limitation in itself does not lead to uncoupling, since a variety of mutant strains exhibit slow-growth phenotypes without associated lethality (4, 7, 36, 44, 47). It appears that HtrB is uniquely involved in the coupling of phospholipid biosynthesis and growth rate under conditions of rapid growth. If the *htrB* mutation primarily affects the LPS layer of the outer membrane, as we have previously proposed (25), the intriguing possibility exists that HtrB provides a link between the regulation of phospholipid biosynthesis, LPS biosynthesis, and bacterial growth.

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EPARATIONS

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[25] Bacterial Lipopolysaccharides

Extraction with Phenol–Water and Further Applications of the Procedure

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Introduction

Liquid phenol is known to be an excellent solvent for many proteins. The partition coefficient in biphasic phenol–water mixtures very often allows an almost complete extraction of proteins from aqueous solutions under controlled conditions of pH and ionic strength in a one-step operation. In contrast, polysaccharides, mucopolysaccharides, lipopolysaccharides, and nucleic acids are usually water-soluble but phenol-insoluble. Various polysaccharides can be precipitated from aqueous solution by adding liquid phenol (see, for example, *1*). Phenol is a weak acid, the dissociation constant at 18–19° in water being $1.1\text{–}1.2 \times 10^{-10}$ (*2*). Mixtures of phenol and water have a high dielectric constant. These facts form the basis of a method of partition of proteins and polysaccharides and/or nucleic acids between phenol and water. Separation of proteins from polysaccharides and nucleic acids by phenol–water is often effected by both the favorable partition coefficient and the dissociation power of phenol–water mixtures.

Morgan and Partridge (*3*) showed that diethylene glycol extracts (endotoxic or whole O-antigenic complex) of various Enterobacteriaceae, such as *Shigella dysenteriae* and *Salmonella typhosa*, are composed of specific polysaccharide, protein, and lipid material (see Vol. V [24]). In 90% liquid phenol solution, the whole complex dissociated. If the reaction mixture was dialyzed against water, the protein precipitated while the undegraded polysaccharide remained in the final water solution. The same method was applied by Goebel and co-workers (*4*) for the

dissociation of the protein–polysaccharide complex from *Shigella sonnei* into phenol-soluble protein and water-soluble lipopolysaccharide. Goebel and Barry (*5*) also used this method for the dissociation of the colicine K-containing complex of *E. coli* K_{235} into protein and lipopolysaccharide.

Palmer and Gerlough (*6*), in attempts to develop suitable direct extraction procedures for enterobacterial somatic polysaccharide antigens, treated whole bacteria with liquid phenol followed by water extraction. Phenol caused the dissociation of the O-antigenic complex in the bacterial cell wall in such a manner that subsequent treatment with water led to the extraction of highly antigenic undegraded polysaccharide of low protein content. For complete polysaccharide extraction, the two-step procedure had to be repeated several times. The Palmer–Gerlough method was also successfully applied in the field of Gram-positive bacteria by M. Heidelberger and co-workers (*7*) for the extraction of capsular polysaccharides from pneumococci; these were then used as powerful antigens in man (*8*).

Westphal and co-workers (*9*) later showed that the Palmer–Gerlough extraction (*6*) can be simplified by shaking bacteria directly in an *emulsion of equal volumes of liquid phenol and water* for a few minutes at low temperature (5–10°). If the mixture is centrifuged, it separates into an upper water layer, a lower phenol layer, and an insoluble residue, the water phase containing the totality of the undegraded polysaccharide (lipopolysaccharide) and nucleic acid (procedure A in ref. *9*). The same procedure was applied by Burton and Carter (*10*) for the extraction of *E. coli O 111* cells; these workers further hydrolyzed the purified protein-free lipopolysaccharide into lipid [lipid A (*11*, *12*)] and degraded polysaccharide. Tauber and Garson (*13*) also made use of the method for the extraction of the endotoxic lipopolysaccharide from *Neisseria gonorrhoeae*. The authors vigorously stirred the mixture in a Waring Blendor for 8 min. During this period, most of the cells disintegrated, and the whole antigenic complex dissociated. The temperature of the mixture rose from about 10° at the beginning to about 40° at the end of stirring. After centrifugation at room temperature, the water and phenol layers were separated. The water phase contained practically all the lipopolysaccharide.

Lipopolysaccharides extracted from bacteria with cold emulsions of phenol–water, however, often still contain varying amounts of firmly bound protein.

At temperatures above 68°, phenol and water are miscible at any proportion (*14*). On cooling, the homogeneous mixture separates into two layers, the upper water phase (saturated with phenol) and the

Procedure

Procedure I (*9, 11, 15, 34*)

Gram-negative bacteria, after cultivation in suitable media, are centrifuged, and the sediment is washed with saline. The bacteria are killed by adding acetone and/or lyophilized from the frozen state.

Phenol–water Extraction

Twenty g. (dry weight) of bacteria, for example, Enterobacteriaceae (*Escherichia, Salmonella,* and so on), are suspended in 350 ml. of water at 65–68° (on a water bath); 350 ml. of 90% phenol, preheated to 65–68°, is then added with vigorous stirring, and the mixture is kept 10–15 min. at 65°. After cooling to about 10° by placing the vessel in an ice bath, the emulsion is centrifuged at 3000 rpm. for 30–45 min., which results in the formation of three layers: a water layer, a phenol layer, and an insoluble residue, the latter sometimes forming a layer at the phenol–water interphase. The water phase is sucked off, and the phenol layer and the insoluble residue are treated at 65–68° with another 350 ml. of water as described above. The combined water extracts are dialyzed 3–4 days against distilled water to remove phenol and small amounts of low-molecular-weight bacterial substances. The dialyzed, slightly opalescent solution, which contains the lipopolysaccharide and ribonucleic acid, is concentrated at 35–40° under reduced pressure to a volume of about 100 ml. After centrifugation for the removal of traces of insoluble material, the water solution is freeze-dried to give an almost white fluffy powder; yield 1.6–2.0 g. (8–10% of the dry weight of the bacteria). The crude extract is composed of about 40–50% of lipopolysaccharide (endotoxic O-antigen) and 50–60% of bacterial ribonucleic acid (RNA).

Removal of Nucleic Acid

The lyophilized crude extract is dissolved in water to give a 3% solution which is centrifuged for 6–8 hr. at 80,000 × g. The sediment is suspended in water, and the suspension is recentrifuged 2–3 times at 105,000 × g. for 3 hr. each. The final sediment is taken up in a minimum amount of water and freeze-dried; yield of bacterial lipopolysaccharide, 300–500 mg. (1.5–2.5% of the dry weight of the bacteria), containing 3% of nucleic acid.

It is known that polyanionic substances form water-insoluble salts with cationic detergents, such as cetyltrimethylammonium bromide ("cetavlon"). However, these salts dissolve in inorganic salt solutions, for example, sodium chloride, the solubility being dependent upon the

ionic strength (and pH) of the medium. For a review see ref. *35*. Using the "cetavlon" technique, Jones (*36*) purified bacterial nucleic acids, and Scott (*37*) fractionated crude heparin preparations and other acidic polysaccharides (Vol. V [11]). On the basis of these results, it was found (*17*) that mixtures of bacterial lipopolysaccharides and nucleic acids, as obtained after phenol–water extraction (Procedure I), can be separated according to the stronger acidic character of nucleic acids in comparison to lipopolysaccharides, which are weakly anionic in character because of their low content of phosphoric acid ester groups (see *11*, *12*, *28*).

This technique can be applied in various ways. (a) The lipopolysaccharide sediment after one or two ultracentrifugations often still contains a few per cent of nucleic acid. By "cetavlon" precipitation, the small amount of RNA can be separated to give an RNA-free lipopolysaccharide (with no absorption maximum at 260 mμ) (Procedure II). (b) The nucleic acid fraction of the supernatant of the ultracentrifuged lipopolysaccharide sediment always contains appreciable amounts of bacterial lipopolysaccharide which, in combination with nucleic acid, does not sediment at 30,000–40,000 rpm. (see above). By "cetavlon" precipitation of the nucleic acid, the remaining lipopolysaccharide can be obtained in purified form. It was found (*17*) that this lipopolysaccharide fraction sometimes differs quantitatively in composition as compared to the first sedimented lipopolysaccharide. For example, the lipopolysaccharide of *E. coli O 111:B4*, prepared according to Procedure I, was found to have a content of 14% of 3,6-dideoxy-L-*xylo*-hexose (colitose, 3-deoxy-L-fucose), while the remaining lipopolysaccharide from the nucleic acid fraction in the supernatant, after "cetavlon" fractionation, had a colitose content of 27–28%. Whether this is an indication for more than one specific lipopolysaccharide in *E. coli O 111:B4* organisms remains to be clarified (see also *37a*). (c) The crude lipopolysaccharide–nucleic acid extract (according to Procedure I) is directly fractionated with "cetavlon" to give the bulk of RNA-free lipopolysaccharide (Procedure III).

Procedure II

One g. of crude lipopolysaccharide, containing 2–5% of RNA, is dissolved in about 150 ml. of water; 15 ml. of a 2% aqueous "cetavlon" solution is added, and the mixture is stirred for about 15 min. at room temperature. The turbid mixture is then centrifuged for 20 min. at 3000 rpm. to remove the precipitated RNA. The opalescent supernatant is lyophilized, and the fluffy residue is dissolved in 50–60 ml. of 0.5 *M* sodium chloride. The solution is poured into a tenfold volume of ethanol

to precipitate the lipopolysaccharide, excess "cetavlon" remaining in solution. After standing 1–2 hr. at 0–4°, the precipitate is centrifuged and redissolved in water. After dialysis for 2 days against deionized water to remove sodium chloride, the solution is freeze-dried (Vol. V [17]); yield about 900 mg. of RNA-free lipopolysaccharide.

Procedure III

The lyophilized crude lipopolysaccharide–nucleic acid extract from the water phase of the phenol–water extraction (Procedure I) is dissolved in 0.5 *M* sodium chloride to give a 0.5–1% solution. A 2% solution of "cetavlon" in 0.5 *M* sodium chloride is added with stirring until the proportion of "cetavlon" to crude extract is about 1.5:1. The solution is now gradually diluted with water, and precipitates are collected by centrifugation as they appear. The RNA–"cetavlon" salt precipitates at a sodium chloride concentration of about 0.3 *M*. The final dilute solution is lyophilized (Vol. V [17]) and taken as the last fraction. The fractions are dissolved in 0.5 *M* sodium chloride and poured into a tenfold volume of ethanol. After centrifugation, the sediment is dissolved in water, dialyzed, and freeze-dried (Vol. V [17]); yield of RNA-free lipopolysaccharide 30–40% of the crude extract.

Fractional "cetavlon" precipitation according to Procedure III proved to be of special value in cases, for example, in the *Salmonella* and *Escherichia* species, in which the water phase after phenol–water extraction, sometimes contained an additional acid mucopolysaccharide in addition to lipopolysaccharide and nucleic acid (*17a*).

Another means of obtaining nucleic acid-free bacterial lipopolysaccharide arose from the finding (*38*) that the phenol–water extraction of formalin-killed *Salmonella* bacteria gives a water phase containing lipopolysaccharide and only small amounts or no RNA. A further investigation (*39*) showed that bacterial RNA, after treatment of the bacteria with diluted formaldehyde (0.1–0.5%), is no longer extractable by phenol–water (Procedure I), probably because cross-linkages are formed between RNA and bacterial protein, giving rise to phenol–water-insoluble complexes. The formalin variation of the phenol–water extraction, however, needs to be investigated in more detail.

Further Applications of the Phenol–water Procedure

Partition of protein and polysaccharide or lipopolysaccharide between phenol and water can be applied for the dissociation and separation of specific precipitates of polysaccharide antigens with antibody (*40*). In principle, the precipitate is dispersed in water, and an equal volume of liquid phenol is added with stirring. After separation of the two phases

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by centrifugation, the water layer contains the antibody-free polysaccharide antigen, which can be isolated and analyzed. The method allows the purification of polysaccharide antigens by aid of specifically precipitating antibodies (*32, 40*); it thus allows the fractionation of polysaccharide antigens according to their serological specificity. Homan and Lens (*41*) purified crude, protein-containing extracts of heparin by partition between phenol and water. From the water phase, which proved to be free of protein, purified heparin could be isolated.

Recently, Broberger and Perlman (*42*) were able to obtain an autoantigen from colonic and other tissues of new-born babies, involved in the pathogenesis of fatal ulcerative colitis. The antigen was extracted with phenol–water at 65° and appeared to be mainly polysaccharide in nature.

On the basis of our findings (*9*), Schramm and co-workers (*43*) developed a method for the dissociation of tabacco mosaic virus (TMV) nucleoprotein into phenol-soluble protein and water-soluble undegraded ribonucleic acid. They were then able to show for the first time that the protein-free TMV ribonucleic acid is the infective unit of the virus. These results prompted a wide application of the phenol–water method to many viruses, and it was clearly shown that the respective nucleic acids acted as the carriers of viral activity. For a review see ref. *44*.

Kirby (*45, 46*) showed that protein-free RNA and DNA (deoxyribonucleic acid) can be extracted from tissues of higher organisms by aid of a modified phenol–water procedure. Normally only RNA is extracted, as with bacteria. If lipophilic and complex-forming salts, such as *p*-aminosalicylate, are added, protein-free DNA can also be extracted. The mixture of RNA and DNA can later be separated by specific precipitation of the DNA fraction.

Nucleic acids (RNA and DNA) of many viruses were extracted from infected tissues, either with cold emulsions or with heated mixtures of phenol and water, and shown to be the infective agents of the virus (as examples, see refs. *47–54*).

From phenol–water dissociated TMV nucleoprotein Anderer (*55*) was able to recover the TMV protein from the phenol solution. The isolated protein recombined with TMV nucleic acid to give crystalline TMV nucleoprotein. This indicates that the protein did not irreversibly denature in liquid phenol. Kickhöfen (*56*) recently demonstrated that various enzymes, ribonuclease, chymotrypsin, trypsin, lysozyme and others, after dissolving in liquid phenol, can also be quantitatively reextracted by a similar technique without loss of enzymic activity. Some enzymes even withstand heating of their phenol solutions up to 80–100°.

The phenol–water extraction, therefore, may probably be not only

applicable for the isolation and purification of polysaccharides, lipopolysaccharides, or nucleic acids, but, in certain instances, also for proteins.

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APPENDIX IV

Cited Statute and Case Law

I. Statute

The first paragraph of 35 U.S.C. § 112 states:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

II. Case Law

Genentech Inc. v. Novo Nordisk A/S, 108 F.3d 1361, 42 U.S.P.Q.2d (BNA) 1001, 1004 (Fed. Cir. 1997).

Decisions of the United States Courts and of the United States Patent and Trademark Office in Patent, Trademark, and Copyright Cases

U.S. Court of Appeals
Federal Circuit

Genentech Inc. v. Novo Nordisk A/S

No. 96-1440

Decided March 13, 1997

PATENTS

1. Patentability/Validity — Specification — Enablement (§115.1105)

Specification of patent in suit would not have enabled person of ordinary skill in art at time of filing to use cleavable fusion expression to make human growth hormone without undue experimentation, since specification merely describes three or four applications for which cleavable fusion expression is generally well-suited, and names enzyme that might be used as cleavage agent as well as sites at which it cleaves, and thus does not describe specific material to be cleaved or any reaction conditions under which cleavable fusion expression would work, and since evidence does not support patentee's contention that disclosure of DNA encoding hGH, combined with prior art cleavable fusion expression techniques applied to non-human proteins, would enable practice of claimed method.

2. Patentability/Validity — Specification — Enablement (§115.1105)

Rule that specification need not disclose what is well known in art means only that omission of minor details does not cause specification to fail to meet enablement requirement, and is not substitute for basic enabling disclosure; if there is no disclosure of any starting material or of any conditions under which claimed process can be carried out, undue experimentation is required, and there is failure to meet enablement requirement that cannot be rectified by asserting that all disclosure related to process is within skill of art.

3. Patentability/Validity — Specification — Enablement (§115.1105)

Specification that states problem of obtaining human growth hormone from precursor containing added protein material does not enable claim for method of producing hGH using cleavable fusion expression, since specification discloses method in which problem is solved by obtaining hGH unaccompanied by leader sequence or other extraneous proteins, but does not provide specific enabling disclosure for obtaining hGH by cleaving hGH-containing protein as recited in claim.

4. Patentability/Validity — Specification — Enablement (§115.1105)

Fact that no one had been able to produce any human protein via cleavable fusion expression as of application date of patent in suit undermines patentee's contention that specification's disclosure of DNA sequence encoding human growth hormone and single example enzyme and its cleavage site, without more, would have enabled one skilled in art to have used claimed cleavable fusion expression method to make hGH without undue experimentation; moreover, if disclosure of useful conjugate protein and method for its cleavage were clearly within skill of art, as patentee asserts, it would have been expressly disclosed in specification, and in customary detail.

Particular patents — Chemical — Human growth hormone

5,424,199, Goeddel and Heyneker, human growth hormone, invalid for lack of enablement.

Appeal from the U.S. District Court for the Southern District of New York, Motley, J.

Action by Genentech Inc. against Novo Nordisk A/S, Novo Nordisk of North America Inc., and Novo Nordisk Pharmaceuticals Inc. for patent infringement. From grant of plaintiff's motion for preliminary injunction, defendants appeal. Injunction vacated; patent held invalid as matter of law for failure of specification to enable practice of claimed method.

Prior decision: 37 USPQ2d 1773.

Leora Ben-Ami, John E. Kidd, Nicholas L. Coch, Joseph Ferraro, Philip E. Roux, and Gerard P. Norton, of Rogers & Wells, New York, N.Y.; Ryan Trainer, of Rogers & Wells, Washington, D.C., for plaintiff-appellee.

Albert L. Jacobs Jr., Jesse D. Reingold, Gerard F. Diebner, Daniel A. Ladow, Brad S. Needleman, and Andrew T. Solomon, of Graham & James, New York; John C. Vassil, Kurt E. Richter, and Kenneth H. Sonnenfeld, of Morgan & Finnegan, New York, for defendants-appellants.

Before Archer chief judge, and Lourie and Bryson, circuit judges.

Lourie, J.

Novo Nordisk A/S, Novo Nordisk of North America, Inc., and Novo Nordisk Pharmaceuticals, Inc. (collectively "Novo") appeal from the order of the United States District Court for the Southern District of New York, issuing a preliminary injunction in favor of Genentech, Inc., enjoining Novo from importing, marketing, using, selling, offering for sale or distributing its Norditropin®-brand recombinant human growth hormone (hGH) product. *Genentech, Inc. v. Novo Nordisk A/S*, 935 F. Supp. 260 (S.D.N.Y. 1996). Because the district court's conclusion that Genentech had demonstrated a likelihood of success on the merits was based on an error of law and because its remaining findings were premised on this error, we vacate the injunction.

BACKGROUND

This consolidated patent infringement action was first brought in the United States District Court for the Southern District of New York on November 30, 1994. On May 12, 1995, Genentech moved for a preliminary injunction under U.S. Patent 4,601,980 to prevent Novo from importing, marketing, using, selling, offering for sale or distributing in the United States its Norditropin®-brand recombinant hGH product. The district court granted Genentech's motion and issued an injunction. *Novo Nordisk of North Am., Inc. v. Genentech, Inc.*, No. 94 Civ. 8634 (CBM), 1995 U.S. Dist. LEXIS 12588, 1995 WL 512171 (S.D.N.Y. Aug. 28, 1995).

On appeal this court vacated the injunction. *Novo Nordisk of North Am., Inc. v. Genentech, Inc.*, 77 F.3d 1364, 37 USPQ2d 1773 (Fed. Cir. 1996). We held that the district court clearly erred in finding that Genentech established a likelihood of proving infringement of the '980 patent because that finding was based on an improper construction of claim 2 of the patent. Based upon the specification and prosecution history, we concluded that because the claim used the phrase "human growth hormone unaccompanied by . . . other extraneous protein," it was limited to processes for directly expressing either hGH or met-hGH. *Id.* at 1371, 37 USPQ2d at 1779. Because the parties agreed that Novo did not use direct expression to produce these proteins, we concluded that Novo did not infringe the patent. *Id.*

Upon returning to the district court, Genentech asserted its newly issued U.S. Patent 5,424,199. The '199 patent has the same specification as the '980 patent and contains a single claim directed to:

> [a] method of producing a protein consisting essentially of amino acids 1-191 of human growth hormone comprising:
>
> (a) expressing in a transformant bacterium, DNA coding for a human growth hormone conjugate protein, which conjugate protein consists essentially of amino acids 1-191 of human growth hormone as set forth in combined Figs. 1 and 3 unaccompanied by the leader sequence of human growth hormone or other extraneous protein bound thereto and an additional amino acid sequence which is specifically cleavable by enzymatic action; and
>
> (b) cleaving extracellularly said conjugate protein by enzymatic action to produce said protein consisting essentially of amino acids 1-191 of human growth hormone.

This claim differs from the claim adjudicated in the prior case in reciting that the encoded protein has an additional amino acid sequence and includes the step of cleaving this conjugate protein. This process of expressing a DNA encoding a conjugate protein and using an enzyme to cleave off an undesired portion of that protein is generally known as cleavable fusion expression. The parties agree that Novo uses cleavable fusion expression to produce hGH. *Id.*

On June 27, 1996, after conducting a twelve-day evidentiary hearing, the district court again issued a preliminary injunction, this time based upon the '199 patent, enjoining Novo from importing, marketing, using, selling, offering for sale, or distributing in the United States its Norditropin®-brand recombinant hGH product. *Genentech v. Novo Nordisk A/S*, 935 F. Supp. 260 (S.D.N.Y. 1996). The district court based its decision upon, *inter alia*, a finding that Genentech would likely overcome Novo's defense that the '199 patent was invalid for lack of an enabling disclosure under 35 U.S.C. § 112, ¶ 1 (1994).

Novo appeals to this court, challenging the grant of the preliminary injunction.[1] We have jurisdiction pursuant to 28 U.S.C. § 1292 (c) (1994).

DISCUSSION

The grant or denial of a preliminary injunction pursuant to 35 U.S.C. § 283 is within the discretion of a district court. *We Care, Inc. v. Ultra-Mark Int'l Corp.*, 930 F.2d 1567, 1570, 18 USPQ2d 1562, 1564 (Fed. Cir. 1991). Accordingly, a trial court's decision granting a preliminary injunction will be overturned on appeal only upon a showing that the court abused its discretion. *Joy Techs., Inc. v. Flakt, Inc.*, 6 F.3d 770, 772, 28 USPQ2d 1378, 1380 (Fed. Cir. 1993). Such an abuse of discretion may be established by showing that the court made a clear error of judgment in weighing relevant factors or exercised its discretion based upon an error of law or clearly erroneous factual findings. *Id.*

As the moving party, Genentech had to establish its right to a preliminary injunction in light of four factors: (1) a reasonable likelihood of success on the merits; (2) irreparable harm if the injunction were not granted; (3) the balance of the hardships; and (4) the impact of the injunction on the public interest. *Nutrition 21 v. United States*, 930 F.2d 867, 869, 18 USPQ2d 1347, 1348-49 (Fed. Cir. 1991); *Hybritech Inc. v. Abbott Lab.*, 849 F.2d 1446, 1451, 7 USPQ2d 1191, 1195 (Fed. Cir. 1988).

A. *Likelihood of Success on the Merits*

In order to demonstrate that it has a likelihood of success, Genentech must show that, in light of the presumptions and burdens that will inhere at trial on the merits, (1) it will likely prove that Novo infringes the '199 patent and (2) its infringement claim will likely withstand Novo's challenges to the validity and enforceability of the '199 patent. *See New England Braiding Co. v. A.W. Chesterton Co.*, 970 F.2d 878, 882-83, 23 USPQ2d 1622, 1625-26 (Fed. Cir. 1992).[2] In other words, if Novo raises a "substantial question" concerning validity, enforceability, or infringement (*i.e.*, asserts a defense that Genentech cannot show "lacks substantial merit") the preliminary injunction should not issue. *Id.* More specifically, with regard to Novo's validity defenses, the question on appeal is whether there is substantial merit to Novo's assertion that the '199 patent claim fails to meet the requirements of 35 U.S.C. § 112, ¶ 1 (1994).

Novo argues that the district court's findings regarding validity under § 112, ¶ 1, are clearly erroneous because it presented clear and convincing evidence that the patent specification would not have enabled a person of ordinary skill in the art to practice the claimed invention without undue experimentation. Novo also argues that the specification fails to contain a written description of the claimed invention. Regarding enablement, Novo argues that the patent is invalid because it does not contain sufficient detail concerning the practice of the claimed method. Novo argues that the mere generic statement of the possibility of cleavable fusion

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[1] On July 3, Novo moved for an emergency stay of the injunction pending disposition of this appeal. On August 1, we denied Novo's motion and reinstated the injunction. However, after having heard oral argument in this case, we reconsidered the motion and reinstated the stay of the injunction.

[2] A patent is presumed valid, 35 U.S.C. § 282 (1994), and a party challenging validity must prove invalidity by clear and convincing evidence. "However, the presumption does not relieve a patentee who moves for preliminary injunction from carrying the normal burden of demonstrating that it will likely succeed on all disputed liability issues at trial, even when the issue concerns the patent's validity." *New England Braiding*, 970 F.2d at 882, 23 USPQ2d at 1625 (citing *Nutrition 21*, 930 F.2d at 869, 18 USPQ2d at 1349).

expression, along with the DNA sequence encoding hGH, a single enzyme (trypsin) for cleaving undisclosed conjugate proteins, and a statement of that enzyme's cleavage sites as being potential amino acid extensions conjugated to hGH is not an enabling disclosure commensurate in scope with the claim. Genentech responds that all of the district court's factual findings regarding enablement are supported by the record. More specifically, Genentech argues that those skilled in the art of recombinant protein expression and purification at the time of filing, July 5, 1979, would have been able to use cleavable fusion expression to produce hGH without undue experimentation by using the teachings of the specification along with methods and tools well known in the art. We conclude that Novo has raised more than a substantial question concerning the validity of the '199 patent. In fact, it has shown that the patent is invalid.

Section § 112, ¶ 1, provides, in relevant part that:

> [t]he specification shall contain a written description of the invention, and the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same. . . .

"[T]o be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation.'" *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993); *see also Amgen Inc. v. Chugai Pharms. Co.*, 927 F.2d 1200, 1212, 18 USPQ2d 1016, 1026 (Fed. Cir. 1991); *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) ("[T]he scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art."). Whether making and using the invention would have required undue experimentation, and thus whether the disclosure is enabling, is a legal conclusion based upon several underlying factual inquiries. *See In re Wands*, 858 F.2d 731, 735, 736-37, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir. 1988).

[1] The question before us is whether the specification would have enabled a person having ordinary skill in the art at the time of filing to use cleavable fusion expression to make hGH without undue experimentation. There is no dispute that the portion of the specification chiefly relied upon by Genentech and by the district court, column 7, lines 29-59, does not describe in any detail whatsoever how to make hGH using cleavable fusion expression. For example, no reaction conditions for the steps needed to produce hGH are provided; no description of any specific cleavable conjugate protein appears. The relevant portion of the specification merely describes three (or perhaps four) applications for which cleavable fusion expression is *generally* well-suited and then names an enzyme that might be used as a cleavage agent (trypsin), along with sites at which it cleaves ("arg-arg or lys-lys, etc.").[1] Thus, the specification does not describe a specific material to be cleaved or any reaction conditions under which cleavable fusion expression would work.

Notwithstanding this limited disclosure, Genentech argues (and the district court found) that those of ordinary skill in the art would have been able to practice the claimed invention without undue experimentation. Essentially, Genentech's argument is that the knowledge of one skilled in the art was sufficient to provide all of the missing information and, more specifically, that the disclosure of a DNA encoding hGH, when combined with prior art cleavable fusion expression techniques applied to non-human proteins, would enable the practice of the claimed method. In support of this argument, Genentech points to the testimony of Dr. Ravetch, who testified as to the knowledge of one skilled in the art, to the extensive description of enzymes in the reference textbook *Methods in Enzymology*, and to the specification's explicit reference to British Patent 2008123-A, which more fully details the potential use of trypsin in cleavable fusion expression.

In response to these arguments, Novo asserts that at the time of filing, trypsin and other like enzymes were used only to digest proteins, not to specifically and precisely cleave conjugate proteins to yield intact, useful proteins, and that the British patent explicitly indicates that trypsin would not be useful for the cleavable fusion expression of arginine-containing proteins such as hGH. Novo further argues that neither the specification nor the references cited by Genentech suggest a single amino acid sequence, out of the virtually infinite range of possibilities, that would yield hGH in a useful form when cleaved from the conjugate protein.

[1] At column 7, lines 52-58, the specification states: "At least in the latter three applications [of the four applications that are disclosed], the synthetic adaptor molecular [sic] employed to complete the coding sequence of the mRNA transcript can additionally incorporate codons for amino acid sequences specifically cleavable, as by enzymatic action. For example, trypsin will cleave specifically at arg-arg or lys-lys, etc."

We agree with Novo. Genentech's arguments, focused almost exclusively on the level of skill in the art, ignore the essence of the enablement requirement. Patent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable. *See Brenner v. Manson*, 383 U.S. 519, 536, 148 USPQ 689, 696 (1966) (stating, in context of the utility requirement, that "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.") Tossing out the mere germ of an idea does not constitute enabling disclosure. While every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification, reasonable detail must be provided in order to enable members of the public to understand and carry out the invention. That requirement has not been met in this specification with respect to the cleavable fusion expression of hGH.

[2] It is true, as Genentech argues, that a specification need not disclose what is well known in the art. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986). However, that general, oft-repeated statement is merely a rule of supplementation, not a substitute for a basic enabling disclosure. It means that the omission of minor details does not cause a specification to fail to meet the enablement requirement. However, when there is no disclosure of any specific starting material or of any of the conditions under which a process can be carried out, undue experimentation is required; there is a failure to meet the enablement requirement that cannot be rectified by asserting that all the disclosure related to the process is within the skill of the art. It is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement. This specification provides only a starting point, a direction for further research.

[3] The specification indicates that it purports to solve a problem. That problem is summarized at column 3, line 65, through column 4, line 8:

> [A] need has existed for new methods of producing hGH and other polypeptide products in quantity and that need has been particularly acute in the case of polypeptides too large to admit to organic synthesis or, for that matter, microbial expression from entirely synthetic genes. Expression of mammalian hormones from mRNA transcripts . . . has permitted only microbial production of bio-inactive conjugates from which the desired hormone could not practically be cleaved.

The problem thus was the difficulty of obtaining hGH from a precursor containing added protein material. This problem was solved by the description of a method of obtaining hGH unaccompanied by a leader sequence or other extraneous proteins, as claimed in the '980 patent. However, the specification for the '199 patent, which is the same as the specification for the '980 patent, does not provide a specific enabling disclosure concerning what the new claim recites, *viz.*, obtaining hGH by cleaving an hGH-containing conjugate protein. That was the problem avoided by the invention claimed in the '980 patent. The present specification contains no more disclosure than the '980 specification, but this patent now purports to claim the unresolved problem that the '980 patent overcame. Genentech is attempting to bootstrap a vague statement of a problem into an enabling disclosure sufficient to dominate someone else's solution of the problem. This it cannot do.

Genentech's arguments in favor of enablement are unavailing. While Genentech's witness, Dr. Ravetch, did state that it would have been possible for a skilled artisan to create a DNA sequence coding for arg-arg-hGH or lys-lys-hGH, he did not discuss the experimentation needed for the creation of DNA coding for more extensive sequences, such as those that have proved necessary to the production of hGH via cleavable fusion expression. Likewise, the description of a wide range of enzymes in *Methods in Enzymology*, by itself, does not render routine the determination of an enzyme-conjugate protein combination. Rather, as Novo argues and the record reflects, various combinations of conjugate protein sequences, cleaving enzymes, and reaction conditions needed to be studied to establish a process for producing hGH in useful form. Finally, the British patent cited in the specification actually works against Genentech's position by explicitly teaching that trypsin would not work well to produce hGH. The specification does not even acknowledge any of the known difficulties associated with using trypsin on an hGH conjugate protein. This specification is so lacking with respect to the limitation of paragraph (b) of claim 1 that providing testimony regarding the skill in the art has been an exercise in futility.

[4] The limited testimony regarding the knowledge of one skilled in the art offered by

Genentech at the preliminary injunction hearing, and relied upon by the district court, is further undermined by the fact that no one had been able to produce *any* human protein via cleavable fusion expression as of the application date. If, as Genentech argues, one skilled in the art, armed only with what the patent specification discloses (a DNA sequence encoding a human protein, in this case, hGH, and a single example of an enzyme and its cleavage site), could have used cleavable fusion expression to make a human protein without undue experimentation, it is remarkable that this method was not used to make any human protein for nearly a year, *see* Shine *et al.*, 285 Nature 456 (June 1980), or to make hGH for five years. *See* Belagaje *et al.*, 3 DNA 120 (1984). Certainly, DNAs encoding desirable human proteins were known at the time of filing (*e.g.*, insulin, described in the British patent), and a great many researchers were attempting to produce human proteins using recombinant DNA technology. This failure of skilled scientists, who were supplied with the teachings that Genentech asserts were sufficient and who were clearly motivated to produce human proteins, indicates that producing hGH via cleavable fusion expression was not then within the skill of the art. The contrary testimony offered by Genentech's witnesses, who hypothesized about the skill of the art more than fifteen years earlier, does not demonstrate the incorrectness of Novo's arguments. *See In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991) ("[A]n expert's opinion on the ultimate legal issue [of enablement] must be supported by something more than a conclusory statement.").

Moreover, it stands to reason that if the disclosure of a useful conjugate protein and the method for its cleavage were so clearly within the skill of the art, it would have been expressly disclosed in the specification, and in the usual detail. Patent draftsmen are not loath to provide actual or constructive examples, with details, concerning how to make what they wish to claim. In addition, as indicated above, the specification of this patent was clearly drafted to claim the invention of obtaining hGH *unaccompanied by* extraneous protein, the cleavage of which was identified by the specification as a problem in this field. Genentech's inventors knew how to enable that which they had invented. These facts underline the inadequacy of the specification in enabling that which it provided only a means to avoid.

The record does not support the district court's implicit finding that the disclosure of trypsin and its cleavage site enables the production of any conjugate protein from which hGH can practically be cleaved and thus produced in useful form; the record indicates that determination of these features required further undue experimentation. None of the expert testimony relied upon by Genentech or by the district court suggests otherwise.[4] Where, as here, the claimed invention is the application of an unpredictable technology in the early stages of development, an enabling description in the specification must provide those skilled in the art with a specific and useful teaching. Genentech has not shown that the '199 patent provides that teaching.

Under the circumstances, we are compelled to conclude that the district court made an error of law in ruling that Genentech showed a likelihood of success on enablement. *See In re Epstein*, 32 F.3d 1559, 1568, 31 USPQ2d 1817, 1823 (Fed. Cir. 1994) ("[E]nablement is a question of law . . . which may involve subsidiary questions of fact."). Furthermore, since we are able to review the record and to read the specification, there is no reason why we should limit our decision here to reversing the grant of the preliminary injunction. Rather, because the parties agreed at oral argument that the enablement issue had been thoroughly ventilated by the extensive arguments before the district court and that court's extensive analysis,[5] we deem it appropriate to rule on the merits of Novo's defense of invalidity. *See* 28 U.S.C. § 2106 (1994) ("The Supreme Court or any other court of appellate jurisdiction may . . . direct the entry of such appropriate judgment, decree, or order, or require such further proceedings to be had as may be just under the circumstances."); *Chicago Observer, Inc. v. City of Chicago*, 929 F.2d 325, 329 (7th Cir. 1991) (reversing preliminary injunction and instructing district court to enter judgment in favor of defendant because the plaintiff "has not suggested that it holds more evidence it could offer at trial and we cannot imagine what additional evidence could aid its cause. Litigation is costly not only for the litigants but also for parties in other cases waiting in the queue for judicial attention. Once it becomes clear that additional proceedings are pointless, the court should bring the case to a close."). We therefore hold that claim 1 and hence the '199 patent are invalid as a matter of law for failure of the specification to enable the practice of the claimed method.

Novo has also argued that the '199 patent is invalid for lack of a written description of the claimed invention and that it is not infringed by Novo. Given our decision on the enablement question, we need not reach these issues.

B. *Other Factors*

Novo also challenges the district court's findings that irreparable harm, the equities, and the public interest favored Genentech. In view of our conclusion concerning the invalidity of the '199 patent, we need not consider these other findings.

CONCLUSION

The court abused its discretion by granting the preliminary injunction based upon an error of law. The district court's error was in finding that Genentech had shown a likelihood of success on the merits since the '199 patent is invalid for failure of the specification to meet the enablement requirement of § 112, ¶ 1. Accordingly, we vacate the injunction and instruct the district court to dismiss Genentech's claim for infringement of the '199 patent on the ground that the patent is invalid.

VACATED.

[4] Novo's witness, Dr. Villa-Komaroff, merely stated on cross-examination that, assuming arg-arg-hGH was initially produced and successfully extracted from the transformed cell, that "[u]nder the best condition, approximately five percent of the time there will be in the [post-digestion] mix [hGH]." This statement, characterized by Genentech as an admission, was made in the limited context of partial trypsin digests of isolated arg-arg-hGH, but none of the necessary experimentation is described in the specification, which is where it should be if it is to contribute to an enabling disclosure.

[5] Genentech stated that it would introduce new evidence at a full trial only in response to new arguments and new defenses raised by Novo. Novo revealed that it had no intention of raising any new arguments or defenses, stating that the "full and complete record" on appeal gave this court "the benefit of everything it really needs" to reach ultimate issues of validity. Thus, considerations that would normally dictate that we limit our decision to reversing the grant of the preliminary injunction are not present. *See University of Texas v. Camenisch*, 451 U.S. 390, 395 (1981) (stating that it is generally inappropriate to render a final judgment on the merits at the preliminary injunction stage because "a preliminary injunction is customarily granted on the basis of procedures that are less formal and *evidence that is less complete than in a trial on the merits.*") (citations omitted) (emphasis added).

U.S. District Court
District of Columbia

U.S. v. The Thomson Corp.

No. 96-1415 (PLF)

Decided December 23, 1996

COPYRIGHTS

1. Elements of copyright — Statutory Elements — Originality (§205.0707)

Legal publisher asserting copyright in "star pagination" of its case law reporters has "thin" copyright claim at best, since, in order to prevail, publisher would have to demonstrate that its reporter page numbers and their placement themselves represent original, creative decision about selection or arrangement, since where and on what pages text of court opinion appears does not embody any original creation of compiler, and since star pagination does not in any way take advantage of that part of publisher's effort in making compilation that reflects its intellectual effort, and instead simply reflects accident of where particular portion of opinion ended up in reporter.

2. Rights in copyright; infringement — Ownership of copyright — Transfer and licensing (§213.0310)

Provision in proposed final judgment in antitrust action, by which two legal publishing companies, as condition of their merger, would be required to grant license for fee to anyone who wants to "star paginate" to case law reporter system, is not in public interest as required by Antitrust Procedures and Penalties Act, 15 USC 16, since copyrightability of star pagination is questionable at best, since including star pagination license in final judgment might be construed as government's endorsement of publishers' dubious copyright claim, since provision would legitimize publishers' ability to profit from licenses while copyright issue is litigated, and since that fact alone is troublesome in view of weakness of copyright claim and limited market power of many of those who would have to pay license fee.

Action brought under federal antitrust laws by the United States and by states of California, Connecticut, Illinois, Massachu-